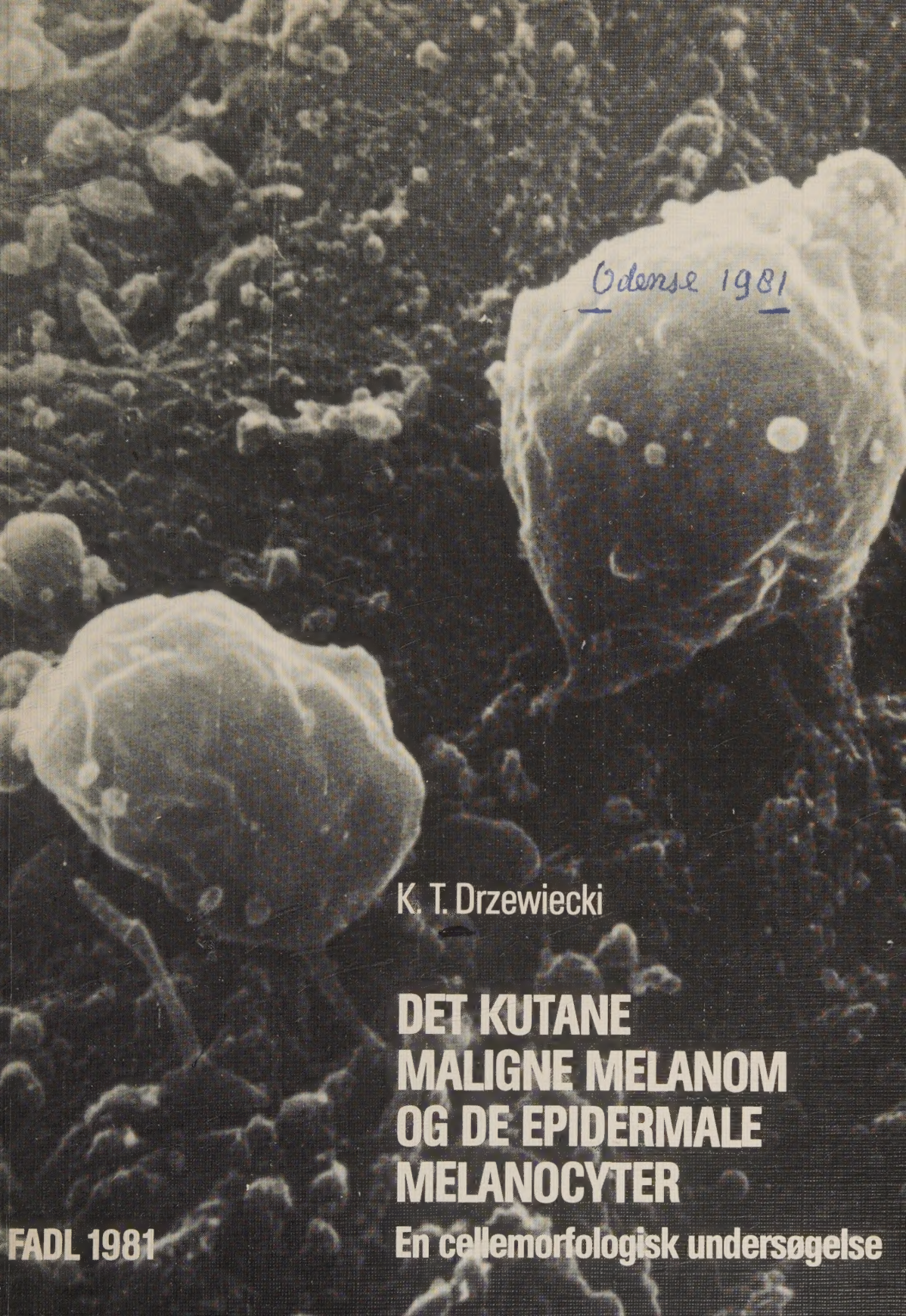


A scanning electron micrograph showing two large, rounded melanocytes in the foreground. They have a granular surface with small, dark, circular features. The background is a dense field of smaller, more irregular cells. The text 'Odense 1981' is handwritten in blue ink on the upper right melanocyte.

Odense 1981

K. T. Drzewiecki

**DET KUTANE
MALIGN MELANOM
OG DE EPIDERMAL
MELANOCYTER**

FADL 1981

En cellemorfologisk undersøgelse

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K. T. Drzewiecki

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OG
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Denne afhandling er den 7. april 1981 af Odense Universitets
lægevidenskabelige fakultetsråd antaget til forsvar for den
medicinske doktorgrad.

Jørgen Ringsted

h.a.dec.



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Forord

Den første inspirerende lektion om maligne melanomer fik jeg i 1966 på Finseninstituttet af overlæge, dr. med. Grete Olsen. Meget senere, i 1974, fik jeg mulighed for systematiske studier af denne hudtumor.

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KAPITEL I

Indledning

Det vides, at det kutane maligne melanom udvikles i det epidermale melanocytsystem. Imidlertid er ætiologien af tumor under udforskning. En række epidemiologiske og kliniske undersøgelser peger på forskellige faktorer, der kan være medvirkende til udviklingen af denne højmaligne tumor. Således har man i visse tilfælde kunnet påvise en genetisk betinget ætiologi (Gellin, Kopf & Garfinkel 1969, Frichot, Lynch, Guirgis, Harris & Lynch 1977, Kopf, Mintzis, Grier, Silvers & Bart 1976, Anderson, Smith & McBride 1967, Anderson 1971, Clark, Reimer, Greene, Ainsworth & Mastrangelo 1978, Wallace, Exton & McLeod 1971, Turkington 1965, Katzenellenbogen & Sandbank 1966, St-Arneault, Nagel, Kirkpatrick, Kirkpatrick & Holland 1970, Shafir & Tsur 1978). Den underliggende genetiske mekanisme er polygenisk, d.v.s. at flere autosomale "gene loci" er involveret i den (Wallace & Exton 1972, Anderson 1972). Videre har man påvist, at f.eks. xeroderma pigmentosum er en prædisponerende lidelse (Moore & Iverson 1964). Det er kendt, at visse virusarter er i stand til at fremkalde maligne tumorer hos mennesker og forsøgsdyr. Det maligne melanom er også blevet undersøgt i denne henseende. Balda & Birkmayer (1973), Parsons, Goss & Pope (1974), Birkmayer, Lubitz, Hammer, Eberhard, Tichmann, Chaussy & Brendel (1976) fandt i de undersøgte humane melanomer tegn på tilstedeværelse af tumorfremkaldende virusarter. Smart & Carle (1975) har beskrevet forekomst af melanomer hos ikke beslægtede ægtepar. Disse forfattere mener, at faktorer i patienternes miljø kan være medvirkende til udvikling af sygdom. Endelig peger de senere undersøgelser på en markant sammenhæng mellem intensiteten af ultraviolet bestråling og forekomsten af det maligne melanom (Mavshovitz & Modan 1973, Wigle 1978, Malec-Olovson 1978, Lee & Merrill 1970, Lee 1972, Beardmore 1972, Fears, Scotto & Schneiderman 1976, Gregg 1977, Houghton, Munster & Viola 1978, Teppo, Pakkanen & Hakulinen 1978).

Undersøgelser af Clark, From, Bernardino & Mihm (1969) og andre undersøgelser af McGovern (1970 & 1976) dannede grundlag for inddeling af maligne melanomer i tre grupper: 1) Lentigo maligna melanomer (LMM), 2) superficielt spredende melanomer (SSM), 3) nodulære melanomer (NM). Clark, Ainsworth, Bernardino, Yang, Mihm & Reed (1975) har isoleret en fjerde form, 4) det uklassificerbare melanom (UM). De tre førstnævnte tumorformer har både forskellige vækstmønstre og forskellig malignitet (Clark et al. 1969, McGovern 1976, Wanebo, Woodruff & Fortner 1975, Cady 1975, McLeod 1975, Søndergård & Hou-Jensen 1977, Woods, Soule & Borkowski 1978, Goldman, Clark, Bernardino & Ainsworth 1976, Eldh, Boeryd & Peterson 1978, Drzewiecki, Christensen, Ladefoged & Poulsen 1980).

Et forsøg på histokemisk differentiering mellem tumortyper blev gennemført af Haensch (1975). I denne undersøgelse blev der påvist forskellig tyrosinaseaktivitet mellem disse tre tumortyper.

På morfologisk basis har undersøgelser af de maligne cellers finstruktur (gennemført af Cesarini 1971, Clark, Heggeler & Bretton 1972, Mishima & Matsunaka 1975, Foa & Aubert 1977) antydnet, at celler i forskellige tumortyper er forskellige. På den anden side har Bleehen, Hartley & Senior (1976) og Hunter, Zaynoun, Paterson, MacKie, Cochran & Bleehen (1976) vist, at kun cellerne i LMM har en rimelig karakteristisk finstruktur. Men sidstnævnte forfattere har ikke kunnet påvise forskelle mellem cellerne i de andre tumorformer. Det er bemærkelsesværdigt, at de anførte undersøgelser udelukkende koncentrerer sig om cellens indre struktur.

Indførelsen af scanning elektronmikroskopi i biologisk forskning intensiverede undersøgelserne af cellernes overflademorfologi. Det viste sig, at forskellige cellearter har morfologisk forskellige overflader. Ydermere er cellens overflade underkastet cykliske ændringer forbundet med cellens biologiske cyklus (Porter, Prescott & Frye 1973, Sauk, White & Witkop 1975). Cellens overflade afspejler ofte cellens aktivitetsniveau (Nabarra, Cavelier, Dy & Dimitriu 1978). En række undersøgelser har påvist, at cellens overflade påvirkes af mange stimuli: 1) morfologien af de epiteliale celler er under konstant indflydelse af hormonal stimulation (Kawada & Naito 1978, Lamb, Newbold, Stumpf & McLachlan 1978, Nathan, Knoth & Nilsson 1978), 2) den påvirkes af ischæmi (Yamada, Talbot, Kobayashi, Shiwaku, Hirakawa, Kosaka & Kimoto 1978), 3) overflademorfologi af

β -cellerne fra pancreas er influeret af D-glukose (Lernmark & Winblad 1977), 4) overfladen af mastocytoma cellerne påvirkes af reaktion mellem overfladereceptorer og Con A (Lustig, Ascher, Fishman, Diodetti & Pluznik 1977), og endelig 5) påvirker også indbyrdes kontakter cellerne imellem deres overflader (Lang, Davidorf, Willis & Sharpnack 1976).

I de senere år er der gennemført en række komparative undersøgelser af maligne el. transformerede celler og tilsvarende normale celler i håb om at finde overflademorfologiske ændringer, der kan være karakteristiske for den maligne celle. Disse undersøgelser blev gennemført på forskellige cellearter både i cellekulturmiljø og i vævsbiopsier. Undersøgelsesresultaterne peger i fire retninger: 1) Porter, Todaro & Fonte (1973), Carley, Moses & Mitchell (1976), Hicks, Newman & Beilby (1977) og Glaser, Mumaw, Farrugia & Munger (1977) har vist, at overfladen af de maligne celler er mere ujævn i forhold til tilsvarende normale celler. Dette er forårsaget af dannelsen af talrige mikrovilli, mikroblebs og blebs, 2) Gondos, Lai & King (1978), Rubio & Kranz (1976), Rubio & Einhorn (1977), Murphy (1977), Friedell, Jacobs, Nagy & Cohen (1977) og Boman, Berg & Christoffersen (1977) mener, at den maligne celled overflade kan karakteriseres ved kvalitative ændringer af de normale cellers kendte overfladeformationer, 3) Faber, Specter & Friedman (1978), og Spring-Millis (1976) har påvist, at overfladen af maligne celler er mere glat i forhold til tilsvarende normale celler. Og endelig 4) har Polliack (1976) påvist, at de maligne celler har samme overflademorfologi som tilsvarende normale celler.

De opnåede uensartede resultater er ikke helt overraskende, idet undersøgelserne blev gennemført på forskellige tumortyper, hvis ætiologi og biologiske opførsel formodes at være vidt forskellig. Yderligere gør Sherman (1977) opmærksom på, at cellerne inden for den samme tumortype (squamocellulært carcinom), som udviklede sig i henholdsvis vagina og ectocervix har forskellig overflademorfologi. Dette synes at være afhængigt af cellernes forskellige embryologiske oprindelse.

Man skal dog understrege, at langt de fleste af de undersøgte maligne celler havde tydelige overflademorfologiske ændringer i forhold til tilsvarende normale celler. En ensartet "malign" overflademorfologisk fenotype blev dog ikke fundet.

Til trods for hurtigt voksende viden om betydningen af tumorcellens overflade for cellens maligne opførsel er der endnu ikke foretaget undersøgelser, der beskæftiger sig med melanomcellens overflademorfologi in situ i biopsier fra en human tumor.

Den foretrukne behandling af det kutane melanom er bred excision af tumor med hud og subkutis. Denne behandling hviler på en lang række kliniske observationer, der viser, at snæver excision af tumor ofte forårsager spredning i form af lokale kutane metastaser. På basis af kliniske og histologiske iagttagelser har Petersen, Bodenham & Lloyd (1962) og Olsen (1966) her fremsat en interessant teori, som går ud på, at det maligne melanom er i stand til at aktivere og eventuelt malignt omdanne de omkringliggende melanocyter. Teorien er tilsyneladende blevet styrket af Wong (1970), Cochran (1971), Seji & Takahashi (1974) og Paul & Gernand (1975), som har påvist både kvantitative og kvalitative ændringer i en population af epidermale melanocyter omkring et malignt melanom. Udstrækningen af disse celleforandringer er af stor betydning for planlægningen af tumors excisionsomfang.

Hovedsigtet med nærværende arbejde er en vurdering af de ovennævnte tidligere arbejder vedrørende melanocytforandringer omkring tumor foretaget på grundlag af en systematisk undersøgelse af de epidermale melanocyter hos patienter med malignt melanom.

Forudsætninger for undersøgelsens gennemførelse har været:

1. Udarbejdelse af en metode, som er egnet til overflademorfologiske undersøgelser af de epidermale melanocyter og maligne melanomceller in situ i hudbiopsier ved anvendelse af scanning elektronmikroskopi.
2. Redegørelse for de normale melanocytters morfologi, funktion og deres regionale fordeling.
3. Komparativ undersøgelse af finstruktur samt overflademorfologi af melanomceller in situ. I forbindelse hermed er cellemorfologien forsøgt relateret til tumortype og klinisk forløb.

KAPITEL II

Materiale og metoder

1. Materiale

Materialet består af hudbiopsier og tumorbiopsier fra 28 patienter med kutant malignt melanom (IV, V, VI og VII) samt af hudbiopsier fra 27 individer, som tjener som kontrolmateriale (I, II og III).

Hos patienter med malignt melanom blev biopsierne taget fra det kirurgiske præparat straks efter excisionen. Der blev taget biopsier fra tumor samt fra tilsyneladende normal hud omkring tumor i henholdsvis få mm, 1 cm, 3 cm, 5 cm og over 5 cm's afstand fra dennes grænse (VI). Desuden blev der taget biopsier fra det anatomisk symmetriske sted, såfremt tumors placering tillod dette. Hos individerne i den anden gruppe blev biopsierne taget peroperativt under forskellige plastikkirurgiske operationer. Disse biopsier blev taget i afstand fra det egentlige operationsfelt.

Hver biopsi blev delt i 4 dele til henholdsvis hæmatoxylin-eosin (HE)-farvning, d-1 dopa (DOPA)-farvning, transmissions elektronmikroskopi (TEM) og scanning elektronmikroskopi (SEM). Hos 16 individer i den anden gruppe udførtes kun DOPA farvning og TEM (III).

2. Metoder

HE-farvning: udførtes på snit fra paraffinblokke efter sædvanlige principper.

DOPA-farvning: Hudbiopsierne inkuberedes i en opløsning af 0,5-2,0 N NaBr i ca. 3 timer. Derefter blev epidermis adskilt fra dermis under dissek-

tionsmikroskop (Fig. 1). De blottede epidermale flader blev inkuberet med DOPA, fikseret, dehydreret og monteret iflg. metode af Staricco & Pinkus (1957). Morfologien af epidermis underside er betinget af tæthed og mønster af hudpapiller. Forsænkninger i epidermis, som svarer til de dermale papiller, blev i artiklerne og i denne oversigt benævnt epidermale dale. Ligeledes blev udfoldninger af epidermis, som svarer til forsænkninger af dermis imellem papillerne, benævnt epidermale rygge (Fig. 2).

Melanocyterne blev morfologisk vurderet og optalt i 4 til 10 tilfældige felter, $0,1089 \text{ mm}^2$ store under $400 \times$ forstørrelse. Antal af optalte melanocyter blev omregnet og udtrykt som antal af celler pr. 1 mm^2 af epitelooverfladen.

Præparation til TEM

Præparaterne (hud/tumorbiopsier eller de blottede epidermale flader ved præparation til SEM-undersøgelse) blev skyllet i phosphate buffer saline (PBS) 2×2 min., fikseret 4 timer i 2,5-3% glutaraldehyd ved 37°C . Derefter blev der foretaget dehydrering i stigende koncentrationer af ethylalkohol, og prøverne blev ført igennem propyleneoxid til indstøbning i Epon 812. Oversigtssnit $1,0 \mu$ tykke og ultrasnit af ca. 500 Å tykkelse blev skåret på LKB ultratom 3. Som elektronmikroskop anvendtes en Hitachi model HS-8.

Præparation til SEM

Biopsierne blev skyllet 2×2 min. i calcium og magnesiumfri PBS ved 37°C og derefter inkuberet i en opløsning af tetrasodium ethylenediamine tetraacetate dihydrate (EDTA) og vævskulturmediet T 199 i ca. 3 timer, ligeledes ved 37°C . Endvidere blev epidermis adskilt fra dermis. De adskilte epidermale og dermale flader blev således separat fikseret i 2,5-3% glutaraldehyd (pH 7,2; 314 mOsm) i 1 time ved 37°C ; herefter skyllet i cacodylatbuffer ved stuetemperatur; - postfikseret $\frac{1}{2}$ time i OsO_4 , ligeledes ved stuetemperatur; - og endelig overført til cacodylatbuffer til opbevaring ved 4°C . Præparaterne blev herefter dehydreret i stigende koncentrationer af acetone 30%, 40%, 50%, 60%, 90% i 15 min. hver, og til sidst 20 min. i 100% acetone. Kritisk-punkt-tørring blev foretaget med kuldioxid, og præparaterne blev monteret på messingcylindre

dels ved hjælp af tape, dels ved hjælp af ledende silverpaint. Præparaterne blev belagt med ca. 200 Å tykt lag af guld og kul i SEM coating unit (E-5000; Polaron Equipment Ltd.). Til undersøgelsen blev anvendt et JEOL JSM-U-2 scanning elektronmikroskop med accelererende spænding fra 16 til 25 KV.

3. Vurdering af metoderne

3.1 DOPA-farvning

Til adskillelsen af epidermis fra dermis anvendtes i forsøgsfasen 4 opløsninger af NaBr. (i) 0,5 N; (ii) 1,0 N; (iii) 1,5 N; (iv) 2,0 N. Der konstateredes ingen bemærkelsesværdig forskel med hensyn til adskillelsetid og præservation af celler i epidermale flader mellem disse opløsninger. 2,0 N opløsning anvendtes dog i langt de fleste tilfælde.

På basis af undersøgelser af over 200 epidermale flader mener jeg at kunne konstatere, at metoden er anvendelig, når følgende fejlkilder tages med i betragtning:

1) Under adskillelsesproceduren kan en del af melanocyterne blive revet ud af epitelet.

2) Nogle celler deformeres ved separationen af epidermis fra dermis.

3) I epidermale flader med høje rygge og dybe dale er det undertiden svært at tælle melanocyterne, idet flere af disse ligger over hinanden på dybe skråninger af ryggene.

4) Fordeling og tæthed af celler i en given epidermal flade er undertiden uensartet, sandsynligvis forårsaget af melanocyternes fysiologiske cyklus.

Disse faktorer skal specielt tages i betragtning ved bedømmelsen af de DOPA-farvede præparater.

3.2 SEM

Scanning elektronmikroskopi er i de senere år forsøgt anvendt til udforskning af forholdene i dermo-epidermal junction. Specielt kan nævnes: Papa & Farber (1971) og Evan, Dail, Dammrose & Palmer (1976). For at kunne

bruge SEM er det nødvendigt at skille epidermis fra dermis igennem lamina lucida oven for den basale membran. Ved udvikling af egen metode har jeg oprindeligt anvendt en modificeret Papa & Farber metode. Modifikationen bestod i, at jeg til adskillelse af epidermis fra dermis anvendte 0,5 N NaBr opløsning i stedet for 2,0 NaBr opløsning. Modifikationen blev foretaget med henblik på at mindske skrumpningsartefakter på overfladen af celler. En 0,5 N opløsning har osmolalitet på 980 mOsm, medens 2,0 opløsning har >1600. Således opnås ved anvendelse af 0,5 N opløsning af NaBr en vis forbedring. Men der var fortsat mange artefakter på overfladen af cellerne, således at det var umuligt at kommentere de forskellige ændringer i overfladens morfologi. En yderligere forbedring af metoden var derfor påkrævet. Til adskillelse af epitelet fra mundslimhinde har Scaletta & McCallum (1972) udarbejdet en metode bestående af inkubering af slimhindebiopsier i EDTA + PBS opløsning. Denne metode viste sig at være velegnet til udforskning af forholdene oven for lamina basalis, idet lamina basalis efter adskillelsen forbliver på dermis (Goel & Jurand 1968). Det vil sige, at de basale epidermocyter med tilhørende melanocyter bliver frilagt. I stedet for EDTA + PBS opløsning har jeg anvendt EDTA + vævskulturmediet T 199. Der er således tale om en modifikation af den oprindelige metode, idet jeg anvender EDTA + T 199 i stedet for EDTA + PBS opløsning. Jeg håber, at cellerne derved bevares levende og intakte under hele adskillellesproceduren (2-3 timer), d.v.s. indtil den endelige fiksering.

Min undersøgelse af talrige epidermale flader førte til følgende iagttagelser:

Mønsteret af dermo-epidermal junction er vidt forskellig i forskellige anatomiske regioner.

I områder med svagt udviklede hudpapillae var epidermis og de tilhørende melanocyter velbevarede (Fig. 3, 4, 5).

På steder med tætte hudpapiller var der en fast forbindelse mellem epidermis og dermis. Adskillelsen i disse tilfælde var vanskelig, og jeg konstaterede tegn på mekanisk skade af såvel melanocyter som epidermocyter (Fig. 6, 7, 8).

I nogle epidermale flader iagttoges hist og her huller i præparatet svarende til udrevne melanocyter. Disse celler blev genfundet beliggende i gruber mellem papillerne på den dermale del af huden.

Strukturen af dermis var i de fleste tilfælde velbevaret.

KAPITEL III

De epidermale melanocyters anatomiske fordeling og deres morfologi

Ref. artiklerne I, II og III.

1. Kvantitative undersøgelser

Optælling af melanocyterne blev foretaget i de DOPA-farvede epidermale flader. I et materiale på 16 individer af skandinavisk afstamning fandtes gennemsnitlig 1548 (± 50) SE melanocyter pr. 1 mm^2 epidermisoverflade. I epidermale flader fra abdomen, dorsum, ansigt og crus fandt vi gennemsnitlig henholdsvis 996, 1278, 2286 og 1564 celler pr. mm^2 af epidermisoverfladen. Signifikante forskelle mellem regionerne hos samme individ ($p < 0,01$) konstateredes. Ligeledes fandtes signifikant forskel mellem individer inden for samme region ($p < 0,01$). Melanocyternes antal afhang af epidermis tykkelse. Det største antal blev påvist i tyk epidermis, og derefter aftog antallet med tykkelsen. Fordeling af melanocyterne i epidermis var vekslende, således fandtes flere melanocyter på skråningerne af de epidermale rygge, men færre i dalene.

2. Bedømmelse af cellernes morfologi

2.1 DOPA-farvning

I de tykke epidermale flader fandtes hovedsageligt runde ovale melanocyter med et vekslende antal dendritter. Dendritterne var som regel fint forgrenede. I de tynde epidermale flader var cellernes udseende stærkt varierende: celler, beliggende på skråninger af epidermale rygge, var ofte ovale og oligodendritiske, hvorimod de melanocyter, som lå i dalene, var større og tillige mere polygonale end de førstnævnte. De udviklede endvidere flere forgrenede dendritter. Desuden påvistes en sammenhæng mellem intensitet af cellernes DOPA-farvning og deres morfologi. Runde og adendritiske celler var i flere tilfælde svagtfarvede. De dendritiske

celler var kraftigere farvede. Omkring flere kraftigt sortfarvede melanocyter fandtes talrige gul-brunfarvede epidermocyter. Imellem svært brunfarvede epidermocyter sås til tider skrumpede blege melanocyter. I nogle tilfælde iagttoges, at de nævnte melanocytforandringer omfattede flere celler i et veldefineret epidermalområde.

2.2 Melanocyternes finstruktur i TEM

Finstrukturen af melanocyterne var varierende. Der blev f.eks. påvist ovale og oligodendritiske celler, enten uden melanosomer eller med få præmelanosomer. Mikrofilamenterne og mikrotubuli var i disse celler svagt udviklede og beliggende centralt i perikarion, i nærheden af kernen. I andre celler sås store mikrofilamentmængder, afgående vinkelret fra kernens overflade og forløbende igennem perikarion og videre ud i dendriterne. I nogle tilfælde iagttoges i nærheden af kernen mikrotubuli, der forløb parallelt med mikrofilamenterne. I de mere dendritiske celler var mikrofilamenterne beliggende perifert, langt fra kerne og i nærheden af Golgi apparat, hvor der fandtes et stort antal melanosomer på forskellige udviklingsstadier. I dendriterne iagttoges talrige mikrofilamenter og mikrotubuli, forløbende parallelt med dendritens længdeakse. Imellem disse fandtes melanosomer. Nogle melanosomer var beliggende i små udposninger på dendriten.

I andre områder af huden sås lyse adendritiske melanocyter omgivet af epidermocyter, der var tungt ladet med melanosomer og melanosomkomplekser.

Vedrørende melanosomernes morfologi blev der iagttaget de velkendte normale modningsstadier (Birbeck & Barnicot, 1959, Drochmans 1966).

2.3 SEM-undersøgelser

Efter adskillelsen af epidermis fra dermis forblev de fleste melanocyter på undersiden af epidermalfladen (Fig. 9). Her fandtes ligesom ved undersøgelsen i DOPA-farvning mange morfologiske varianter. I de fleste tilfælde var overfladen glat på de dendritiske celler, hvorimod de runde adendritiske celler snart frembød en ujævn og snart en glat overflade. Den ujævne overflade var besat med udposninger i form af mikrovilli, blebs og mikroblebs. I nogle tilfælde kunne der iagttages mikrovilli udgående såvel fra melanocyt som fra epidermocyt (Fig. 10). Dendriterne,

der som regel efter et kort forløb i dermo-epidermal (de) junction forsvandt imellem basal-epidermocyterne, viste sig at afgive sidegrene. Disse forsvandt ligeledes i de fleste tilfælde imellem epidermocyterne (Fig. 11). Nogle af dendriterne havde fusiforme udvidelser langs deres forløb og udposninger i form af pærelignende sideskud. I få tilfælde iagttoges, at dendritspidsen forsvandt i en tragtliggende formation på epidermocyten's overflade (Fig. 13). I nogle præparater kunne iagttages, at dendriterne berørte overfladen af enten epidermocyt eller melanocyt. Dog sås ikke specialiseret kontaktorganel. I intet tilfælde påvistes et netværk af indbyrdes kommuniserende dendriter i d-e-junction.

3. Diskussion

Et gennemsnitligt antal af melanocyter hos vores 16 individer af skandinavisk afstamning viste sig at være i overensstemmelse med Szabo's undersøgelser (Szabo 1959, 1967) og ligger således inden for normalområdet for individer uden forstyrrelser i hudpigmenteringen. I overensstemmelse med Staricco & Pinkus; Mitchell (1963), Szabo (1967), Pinkus, Staricco, Kropp & Fan (1959), Hu, Staricco, Pinkus & Fosnaugh (1957) påviste vi, at antallet af epidermale melanocyter viste signifikante forskelle mellem anatomiske regioner ($p < 0,01$). Dette forhold afspejler afgjort hudens varierende funktioner i forskellige anatomiske regioner og det differentierede behov for melaninpigment. Bortset fra interregionale forskelle har vi således påvist signifikante individuelle forskelle, som med overvejende sandsynlighed er genetisk betingede.

I et materiale på 27 individer gennemførte vi cellemorfologiske studier. De epidermale melanocyters morfologi viste her en interessant variation. Cellemorfologiske variationer blev også påvist af Staricco & Pinkus; Mitchell; Szabo (1967), Glimcher, Kostick & Szabo (1973); Quevedo, Szabo, Virks & Sinesi (1965). En gennemgang af talrige melanocytformer sammenholdt med cellernes finstruktur og forskellig farvbarhed med DOPA har ved denne undersøgelse givet mig en formodning om en cyklisk melanocytfunktion. Fitzpatrick, Hori, Toda, Kinebuchi & Szabo (1971), Jimbow, Pathak & Fitzpatrick (1973) har beskrevet en såkaldt "inactive melanocyte", eller som den også betegnes den "DOPA-negative melanocyte", (Szabo 1976, Quevedo et al.; Mishima & Widlan 1967). Det må formodes, at cyklus begynder med ovenfor nævnte celleform, som er oval, adendritisk, meget svagt

DOPA-farvet og indeholdende få præmelanosomer. Cellens mikrofilament-mikrotubuli system er ligeledes svagt udviklet. Cellens aktivitet begynder sandsynligvis med udvikling af cytoskelettet (mikrotubuli og mikrofilamenter). Et velfungerende cytoskelet må formodes at være en betingelse for det dendritiske vækstmønster, som blev påvist såvel i de DOPA-farvede præparater som ved SEM. Cytoskelettet er ligeledes nødvendigt for den intracellulære transport af melanosomer (Magun 1973, McGuire, Moellmann & McKenon 1972, Jimbow et al., Moellmann, McGuire & Lerner 1973, Jimbow & Fitzpatrick 1975 og McGuire & Moellmann 1972. Udviklingen af cytoskelettet ledsages af stigende melanogenese, d.v.s. dannelsen og modningen af flere melanosomer. Ved hjælp af cytoskelettet (mikrofilamenter) bliver disse transporteret ud i dendriterne (Jimbow & Fitzpatrick, Jimbow et al.) for derefter at blive overført til epidermocyterne (Wikswø & Szabo 1972). Overføringsmekanismen er ikke universelt accepteret. I én teori hævdes det, at melanocyten aktivt sprøjter melanosomer ind i epidermocyterne (Masson 1948, Drochmans 1961 og Mishima 1966). I en anden hævdes det, at melanosomerne udskilles i det intercellulære rum og derfra optages af epidermocyterne (Swift 1964, Wolff, Jimbow & Fitzpatrick 1974, Kukita, Sato & Ito 1974 og Wolff 1973). Endelig er der en tredje teori, som går ud på, at epidermocyten fagocyterer et stykke af melanocyten dendrit, som er fyldt med melanosomer (Cruickshank & Harcourt 1964, Mottaz & Zelickson 1967 og Okazaki, Uzuka, Morikawa, Toda & Seiji 1976). Vores undersøgelser giver ikke entydige beviser til belysning af de nævnte teorier. Vi har dog iagttaget, at melanosomer ophobes i pærelignende udposninger på dendriten, og at dendritspidserne i nogle tilfælde bliver omringet af tragtlignende formationer på epidermocyternes membran. Disse iagttagelser kan sandsynliggøre den cytofagocytotiske teori nr. 3.

Ud fra vores observationer er det sandsynligt, at melanocyten skrumper ind og går over i en hvilende form "dormant melanocyte" efter overførslen af melanosomer. Undersøgelsen giver ikke svar på, om ovenfor beskrevne melanocytcyklus har nogen relation til melanocyternes mitotiske aktivitet. Jeg har ikke iagttaget melanocyter i mitose. Den anvendte undersøgelsesmetode belyser således heller ikke varigheden af den beskrevne cyklus.

Pinkus et al. er af den opfattelse, at melanocyter ikke påvirkes af epidermocyternes mitotiske aktivitet, medens Hadley & Quevedo (1966) og Fitzpatrick & Breathnach (1963) hævder, at ultraviolet lys stimulerer

melanogenesen indirekte ved at inducere epidermocytoproliferationen. Selv vurderer jeg på basis af mine undersøgelser, at melanocytenes cykliske funktion er relateret til epidermocytenes mitotiske aktivitet, idet melanocyten skal forsyne en ny population af epidermocyter med melanosomer. Dette antyder en afhængighed mellem de to celletyper i epidermis.

Fitzpatrick & Breathnach har fremsat en teori om, at melanocyten sammen med et vist antal af epidermocyter danner en funktionel enhed, benævnt "epidermal melanin unit". Denne teori er blevet bekræftet af Frenk & Schellhorn (1969), og mine kvantitative og kvalitative studier af melanocyter peger i samme retning. Under en normal fysiologisk tilstand er antallet af epidermocyter pr. melanocyt ret konstant (Szabo 1959, 1967, Fitzpatrick & Breathnach og Fitzpatrick et al.). Det kan således antages, at, når antallet af epidermocyter stiger, vil antallet af melanocyter også gå op. Der blev således i mit materiale fundet flere melanocyter i tyk epidermis end i tynd epidermis. Melanocyt/epidermocytt forholdet afspejler sig dermed ved melanocytenes fordeling og morfologi imellem epidermale rygge og dale. Der er flere epidermocyter i de epidermale rygge, og disse er mere aktive. Svarende hertil kunne der også påvises flere melanocyter. En epidermocytpulje, som tilhører en bestemt melanocyt, vil i de epidermale dale strække sig over større område. Af samme grund er melanocyterne her polygonale og udvikler lange forgrenede dendritter (Fig. 14). Den beskrevne kvantitative fordeling, sammenholdt med melanocytenes morfologi, bekræfter teorien om en funktionel afhængighed mellem disse celler i epidermis. Ydermere har jeg i nogle tilfælde påvist dannelsen af mikrovilli både udgående fra melanocyt og epidermocytt. Det er muligt, at disse mikrovilli repræsenterer en kontaktorganel inden for den epidermale melaninenhed.

KAPITEL IV

Det maligne melanom - en cellemorfologisk undersøgelse

Ref. artiklerne IV og V.

1. SEM-undersøgelse

1.1 Tumors vækstmønster

To vækstformer kunne iagttages: 1) diffus infiltrerende vækst (Fig. 15, 16) og 2) vækst i form af cellereder (Fig. 17, 18). De nodulære melanomer var oftest karakteriseret ved diffus infiltrerende vækst, medens de superficielt spredende melanomer frembyder begge former for tumorvækst.

1.2 Den maligne celled morfologi

Det maligne melanom fremtræder som en polymorf tumor, hvad angår celledernes form og morfologien af deres overflader. Der konstateredes forekomst af tre celleder: runde/ovale celler (Fig. 16, 17), dendritiske celler (Fig. 15) og tenformede celler (Fig. 18). De 3 celleder var varierende, hvad størrelse og overflademorfologi angik, men der kunne dog iagttages en vis tendens. De runde/ovale celler havde oftest en mere ujævn overflade end de aflange dendritiske celler. Cellerne, der fandtes i den invasive rand af tumor eller i den ulcererede overflade, havde en stærkt foldet og uregelmæssig overflade med dannelse af blebs, mikrolebs og mikrovilli. I biopsier fra såvel SSM som NM kunne iagttages løst liggende celler eller cellereder, som ved mindste vipning af præparatblokken flyttede sig. Dette gjaldt navnlig runde blebbede celler (Fig. 17).

2. TEM-undersøgelse

2.1 Den maligne celled finstruktur

De fleste celler havde forøget kerne/cytoplasma ratio. Kernernes konturer

var stærkt foldet, og i flere celler var kernerne multilobære (Fig. 19). Nukleoli var store og med groft kromatinindhold og i flere celler multiple. I alle maligne celler var antallet af hypertrofiske mitochondrier forøget, og der sås mange bizarre former. Golgi apparatet, det endoplasmatiske retikulum, ribosomer og polyribosomer dominerede ofte endoplasmaet. Alle melanomceller var forsynet med melanosomer med strukturelle afvigelser fra normale melanosomer. På grundlag af deres strukturelle opbygning kunne de inddeles i fibrillære, granulære og vesikulære melanosomer. Det skal dog understreges, at alle tre strukturer (fibrillær, granulær og vesikler) kunne forekomme inden for det samme melanosom. Såvel aflejningsmønsteret som intensiteten af det i melanosomerne forekommende melanin varierede stærkt og bidrog således til yderligere variation i melanosomets morfologi. Melanosomernes antal var også varierende fra celle til celle. En nøje gennemgang af melanosomerne gjorde det dog muligt at identificere celler, der ligner hinanden, d.v.s. tilhører den samme cellelinje. Inden for samme tumor optræder ofte 2 til 3 forskellige cellelinjer.

Hos fire ud af 22 patienter fandtes melanomceller, som var omgivet af en zone med fintgrynet substans, hvori antal af de kollagene fibriller tydeligt var mindsket. Disse celler havde som regel en stærkt ujævn overflade (Fig. 20, 21).

2.2 Mikrotubuli og mikrofilamenter i melanomcellen

Mikrotubuli fandtes i de fleste maligne celler. Antallet af dem varierede stærkt i forhold til antallet i de normale melanocyter hos samme patient. I mange melanomceller kunne der dog påvises et tilsyneladende normalt mikrotubulisystem.

Der fandtes ligeledes mikrofilamenter i samtlige maligne celler, og her var antallet også varierende i forhold til de normale melanocyter hos samme patient. Forløbet af mikrofilamenterne i de maligne celler var tilsyneladende helt tilfældigt, uanset om de var anordnet i bundter eller forløb enkeltvis, og var således stærkt varierende i forhold til normale melanocyter hos samme patient. I nogle ovale celler fandtes kæmpe-mikrofilamentkomplekser samlet et enkelt sted i cytoplasmaet i form af en ø.

2.3 Cellens overflademorfologi relateret til dens mikrotubuli og mikrofilamenter

I nogle celler fandtes en vis sammenhæng mellem cellens overflade og dens cytoskelet. I celler med glat overflade var mikrotubuli orienteret parallelt med overfladen. Andre celler med ujævn overflade havde mikrotubuli orienteret skråt eller vinkelret på denne. Af og til observeredes mikrotubuli beliggende i villi og blebsformationerne. Endelig, hvad angik mikrofilamenterne, var disse i celler med ujævn overflade ofte beliggende tilfældigt sammenslynget under cellemembranen.

2.4 Den maligne celledens finstruktur korreleret med nogle af tumors histologiske og kliniske aspekter.

I syv ud af 23 primærtumorer fandtes celler med multilobære kerner. Fem af disse tumorer var NM og to SSM. Alle her nævnte nodulære melanomer metastaserede enten til de regionale lymfeknuder eller til huden. Ingen af de SSM metastaserede. Fire af de fem patienter med nodulære melanomer døde af sygdommen. Disse patienters tumorer var opbygget af celler med et påfaldende stort mikrofilamentindhold. Det var ikke muligt på basis af melanosomernes udseende at differentiere mellem SSM og NM.

Det konstateredes, at de dermale metastaser og lymfeknudemetastaserne var opbygget af morfologisk samme tumorceller eller cellelinjer, som morfologisk var identiske med dem, der oprindeligt blev fundet i den primære tumor.

3. Diskussion

Den normalt differentierede melanocyt er i besiddelse af den helt specielle egenskab, at den er i stand til at producere pigmentgranula, de såkaldte melanosomer. Melanosomerne har en karakteristisk og veldefineret struktur (Birbeck & Barnicot, Drochmans; Hunter, Mottaz & Zelickson 1970, Jimbow & Fitzpatrick 1973, Breathnach, Gross & Martin 1973 og Allegra 1974). Finstrukturen af melanosomerne i de fleste maligne celler ændres betydeligt. De morfologiske forandringer må anses som værende en ændring i forskellige faser i melanosomdannelsen. Disse faser omfatter opbygning af melanosomets proteinmatrix samt aflejring af melanin med vekslende intensitet og aflejringssmønster (Cesarini, Clark et al. 1972, Clark 1973, Curran & McCann 1976, Hunter, Paterson & Fairley 1978a, Hunter, Zaynoun,

Paterson, Bleehe, Mackie & Cochran 1978b og Drzewiecki jf. artikel IV).

Melanosomerne er af Clark et al. 1972 blevet opdelt i normale melanosomer, abortive melanosomer, lamellære melanosomer og granulære melanosomer med det formål at differentiere mellem de maligne celler. Jeg har forsøgt at anvende Clark et al.'s klassifikation og kan konstatere, at kun normale og granulære melanosomer kan genfindes. Der forekom så stor overlappning mellem abortive og lamellære melanosomer, at det for mig var vanskeligt at differentiere mellem disse.

Derudover har jeg konstateret forekomst af melanosomer opbygget af vesikler og har benævnt denne form: vesikulære melanosomer. Jeg var ikke i stand til at påvise de af Mishima (1967) og Klug & Günther (1972) beskrevne cigarformede melanosomer.

Konkluderende kan jeg anføre, at i maligne melanomceller forekommer tre strukturelle melanosomformer: fibrillære melanosomer (hvor normale, abortive og lamellære melanosomer indgår; eftersom de alle tre har en fibrillær struktur), granulære melanosomer og vesikulære melanosomer. Endvidere skal jeg nævne, at der ofte forekommer overlappning mellem disse melanosomformer inden for samme celle.

På basis af melanosomernes ultrastruktur sammenholdt med ændringerne i cellens andre organeller har Clark et al. 1972 og Hunter et al. 1978 opdelt cellerne i 4 grupper, hvor celledifferentiering aftager fra den højt differentierede gruppe 1 til den lavt differentierede gruppe 4. Foa & Aubert har foretaget yderligere opdeling af melanomcellerne og differentierede dem i hele 5 grupper.

Den tidligere nævnte melanosompolymorfi, som oftest er til stede i melanomcellerne, vanskeliggør den af Clark et al. (1972), Hunter et al. (1978a) og Foa & Aubert foreslåede celleklassificering og gør den usikker. En opdeling af cellerne i blot to grupper: højtdifferentierede celler og lavtdifferentierede celler, vil gøre inddelingen mere anvendelig. Cellerne, hvori der gennemgående findes hypertrofiske, fibrillære melanosomer af enten helt normal struktur eller visende lettere ændringer i form af manglende "crossing over" af melanosomfibrillerne, er højtdifferentierede. Cellerne, hvori der findes talrige andre melanosomvarianter,

hører til gruppen: lavtdifferentierede celler.

I de normale melanocyter er den intracellulære transport og udskillelsen af melanosomerne afhængig af et velfungerende mikrotubulus- og mikrofilamentsystem (III). Melanosomerne i de maligne celler er tilfældigt placeret i cytoplasmaet, helt uafhængigt af deres modningsgrad. Desuden findes talrige melanosomer beliggende frit uden for cellerne eller fagocyteret af makrofager, et forhold, der sandsynligvis er betinget af en ændring i den intracellulære transport og i udskillelsesmekanismen af melanosomerne fra de maligne celler. Det var derfor nødvendigt at undersøge morfologien af mikrotubuli og mikrofilamenter i melanomcellen.

Deres betydning blev accentueret yderligere i de af Nicolson, Poste & Tae (1977a), Albertini & Anderson (1977), Berlin, Oliver, Ukena & Yin (1975), Albertini & Clark (1975), DeBrabander, DeMey, Van de Veire, Aerts & Geuens (1977), Nicolson & Poste (1976) foretagne undersøgelser, gennemført på forskellige cellearter i cellekultur. Disse undersøgelser gav formodning om, at cellens form, overflademorfologi og dens egenskaber er reguleret via mikrotubuli og til en vis grad også mikrofilamenter (Albertini & Anderson, Haemmerli & Felix 1977).

Transformeringen af flere celletyper i cellekulturer blev ledsaget af morfologiske ændringer i deres mikrotubulikomplekser (Fuller & Brinkley 1976 og Brinkley, Fuller & Highfield 1975). Der findes dog også undersøgelser, konkluderende at mikrotubulikomplekserne var upåvirkede ved transformeringen (DeMey, Joniau, DeBrabander, Moens & Geuens 1978 og Osborn & Weber 1977). Lantos (1977) har i eksperimentelt inducerede maligne gliomer hos rotter påvist ændringer i de maligne astrocyters mikrotubuli og mikrofilamenter. Flere undersøgelsesresultater tyder på, at ændringer i den transformerede celledes mikrotubulisystem ledsages af ændringer i cellens overflade, såvel hvad angår dens morfologi som dens egenskaber (McClain & Edelman 1978, Haemmerli & Felix). Ændringer i cellens mikrofilamenter, mikrotubuli, overflademorfologi og dens egenskaber har sandsynligvis afgørende betydning for cellens maligne opførsel, d.v.s. ukontrolleret proliferation, løsrivelse fra primærtumor, invasion i omgivende væv og metastasering (Nicolson 1977 (a review), Mareel & DeBrabander 1978, Poste 1977 (a review), Pourreau-Schneider & Sträuli 1977, Miller, Fuseler & Brinkley 1977, Tao & Burger 1977, Brunson, Beattie & Nicolson 1978 og

Nicolson, Birdwell, Brunson, Robbins, Beattie & Fidler 1977b). Disse konklusioner er hovedsagelig baseret på eksperimenter med kulturer af forskellige cellelinjer, gennemført in vitro og in vivo (på forsøgsdyr). Det var derfor ønskeligt at undersøge morfologien af maligne cellers mikrotubuli, mikrofilamenter og deres celleoverflader in situ i biopsier fra humane tumorer.

Mikrotubuli påvistes i de fleste melanomceller. I de fleste celler fandtes ingen markante ændringer i mikrotubulis forløb og placering i cytoplasmaet sammenlignet med normale melanocyter hos den samme patient. I nogle tilfælde kunne placeringen af mikrotubuli dog relateres til morfologien af cellens overflade. Ovale celler med ujævn overflade havde en del mikrotubuli forløbende vinkelret på denne. En del fandtes beliggende i udposninger såsom blebs, mikroblebs og mikrovilli. De dendritiske celler med glat overflade havde mikrotubuli forløbende parallelt med overfladen.

Alle maligne melanomceller var forsynet med mikrofilamenter. Såvel deres mængde som anordning og placering i cytoplasmaet afveg fra det, man så i normale melanocyter hos den samme patient. De mest accentuerede forandringer i form af ansamlinger af sammenhængende mikrofilamenter fandtes oftest i de ovale adendritiske celler.

Undersøgelsesresultaterne viste, at såvel mikrofilamenter som mikrotubuli i de maligne celler var ændret kvantitativt. Desuden sås, hvad angik mikrofilamenterne, morfologiske ændringer. Disse ændringer må anses for at være udtryk for en ustabilitet i cytoskelettet. Dette ustabile system er formentlig ansvarligt for den ændrede transport og udskillelse af melanosomer fra den maligne celle. Desuden influerer det sandsynligvis på såvel celleform som overflademorfologi. Morfologiske ændringer i mikrofilamenterne og mikrotubuli fandtes således oftest i de ovale celler, som i øvrigt hyppigst havde ujævn overflade.

Ovale celler var oftest beliggende i den invasive rand af tumor og i tumors ulcererede områder. Disse cellers morfologi sammenholdt med beliggenhed i tumor giver mistanke om, at de udgør en celleform, der aktivt invaderer værtsorganismen, idet González-Cámpora, Haynes & Weller (1978) og Karnovsky & Unanue (1978) mener, at forekomst af overfladestrukturer

såsom mikrovilli, mikrolebs og ruffles sammen med ændringer i deres kvantitative fordeling over cellens overflade er et tegn på motilitet af cellerne.

Hashimoto, Yamanishi, Maeyns, Dabbous & Kanzaki (1973), Sylven (1973) og Strauch (1972) har påvist, at nogle højt maligne tumorer er i besiddelse af "cellreleased" eller "cellesurfacebound" enzymer, som er i stand til at nedbryde den omgivende grundsubstans. Steigleder & Uerdingen har påvist, at mængden af det kollagene væv er formindsket i omfanget af de maligne melanomer. Ved TEM undersøgelser har jeg hos fire patienter påvist, at melanomcellerne, der var beliggende i tumors invasive rand, i nogle tilfælde var omgivet af en zone med fintgrynet substans og et formindsket antal af kollagene fibriller. I øvrigt var overfladen af disse celler ujævn. Ved en gennemgang af cellens organeller fandtes små vesikler beliggende i cytoplasmaet i tæt relation til cellemembranen (Fig. 21).

Dette kan antyde, at nogle af melanomcellerne frembyder et morfologisk tegn på kollagenolytisk aktivitet. Denne aktivitet kunne tænkes at være af betydning for cellens invasionsevne i grundsubstansen.

Ved SEM undersøgelser af flere tumorbiopsier iagttoges, at cellerne eller mindre cellereeder ved vipning af præparatblokken ofte kunne flyttes. Dette kan betragtes som tegn på formindsket adhæsion mellem cellerne. Coman (1944) og Weiss (1967) har konstateret, at de adhæsive kræfter mellem cellerne er formindsket i de maligne tumorer, og at dette bl.a. kan spille en rolle for cellernes løsrivelse fra selve tumor.

Ved alle anvendte mikroskopiske metoder konstateredes, at de fleste maligne melanomer var opbygget af to til tre morfologisk forskellige cellelinjer. Det kan således bekræftes, at Clark et al.'s (1975) påstand om, at der i maligne melanomer forekommer "intralesional transformation", der resulterer i opståen af flere forskelligt differentierede cellelinjer, er gældende. Der fandtes ingen markant forskel mellem SSM og NM med hensyn til optræden af højtdifferentierede og lavtdifferentierede celler.

Hver af de undersøgte metastaser var opbygget af morfologisk samme celler eller cellelinjer, som fandtes i primærtumor. Det må således fremføres,

at melanomcellerne er i stand til at bevare deres morfologiske fenotype til trods for indflydelse af andre celler i dermis, lymfesystem og blodbaner. Undersøgelserne giver ikke svar på, i hvilken udstrækning cellernes differentieringsgrad kan korreleres med deres metastaseringsvejen. Det er ikke overraskende, idet der findes mange andre faktorer, som spiller en rolle i metastaseringsprocessen.

Over 90% af de kutane melanomer kan placeres inden for tre hovedgrupper: LMM, SSM og NM (Mastrangelo, Clark, Bellet & Berd 1978). Disse tumortyper har forskellig malignitet. Ultrastrukturelle studier af LMM tyder på, at det er en særskilt tumorform (Hirone, Nagai, Matsubara & Fukushima 1971, Lupulescu, Pinkus, Birmingham, Usdek & Posch 1973, Bleeher et al.). Undersøgelser af Clark et al. (1972) har yderligere vist, at der også mellem SSM og NM findes ultrastrukturelle forskelle. Imidlertid har Hunter et al. (1978a) ikke kunnet påvise forskelle mellem de to sidstnævnte tumortyper. Ved undersøgelse af melanosomer, mitochondrier, Golgi apparat, det endoplasmatiske retikulum og celleoverfladerne i talrige celler fra både SSM og NM har jeg heller ikke kunnet finde forskel mellem cellerne i de to tumortyper.

Nogle af tumorerne var opbygget af celler med stort mikrofilamentindhold. Det vides, at bl.a. mikrofilamenterne spiller en afgørende rolle i cellens bevægelighed og formændringer (Haemmerli & Felix). Ydermere har Felix & Sträuli (1976) påvist, at mikrofilamenterne er anordnet forskelligt i henholdsvis hvilende og mobile leukæmiske celler. Komparative TEM undersøgelser af to leukæmiske celletyper, som karakteriseres ved henholdsvis stor bevægelighed og beskeden bevægelighed, viste markant forskel i mængden af mikrofilamenter. De førstnævnte celler havde store mængder af mikrofilamenter, hvorimod de sidstnævnte kun havde enkelte (Haemmerli et al.). Yderligere påvistes, at de mere bevægelige celletyper havde betydeligt større invasionsevne end de mindre bevægelige leukæmiske celler (Pourreau-Schneider & Sträuli). Det forekommer logisk, at cellens øgede bevægelighed og invasionsevne vil spille en betydelig rolle i metastaseringsprocessen. Det blev således i mit materiale påvist, at det kliniske forløb af maligne melanomer tildels kunne korreleres med mængden og anordningen af mikrofilamenterne i cellerne. Disse tumorer, som metastaserede, var opbygget af celler, som indeholdt betydelige mængder af sammenslyngede mikrofilamentbundter placeret tilfældigt i cytoplasmaet.

KAPITEL V

Det epidermale melanocytsystem hos patienter med malignt melanom

Ref. artiklerne VI og VII.

1. Kvalitative undersøgelser

1.1 DOPA-farvning

Formålet med denne undersøgelse var at bedømme melanocyter med henblik på evt. iagttagelse af suspekterte celleformer samt at påvise evt. aktivering af melanocyter og dennes omfang.

Hos 8 ud af 22 patienter fandtes i epidermis, i umiddelbar nærhed af tumor, suspekterte melanocytformer og enkelte maligne celler. De førstnævnte celler var store og sortfarvede, nogle af dem ovale. Atter andre udviklede stumpe klodsede dendritter (Fig. 22). Morfologi af maligne celler svarede til morfologien af melanomcellerne i primærtumor (Fig. 23). Fem af de 8 patienter havde SSM og tre NM. Aktivering iagttoges hos 5 ud af 22 patienter. Her viste det sig, at kun melanocyter nær tumor aktiveredes, d.v.s. en afstand på få millimeter op til 1 cm. Melanocyterne var brun-sort farvede, større end de inaktiverede celler, og de udviklede flere forgrenede dendritter (Fig. 24). Enkelte af cellerne var kraftigt hypertrofiske med dendritspidser omringet af utallige melanosomer, som tilsyneladende var beliggende uden for cellerne (Fig. 25). Interessant nok fandtes der aldrig aktivering af melanocyterne hos patienter med nodulære melanomer.

1.2 TEM-undersøgelse

Hos 12 ud af 27 patienter iagttoges melanocyter med kæpemelanosomkomplekser. Disse celler var hyppigst beliggende i nærheden af primærtumor, men de fandtes også, dog med lavere hyppighed, i større afstande fra tumor.

Cellerne påvistes ligeledes i kontrolbiopsierne. I nærheden af tumor hos 6 ud af 27 patienter sås Langerhans'ske celler og fibrocyter med melanosomkomplekser beliggende i deres cytoplasma. Fem patienter havde melanocyter med makromelanosomer af enten globulær eller fibrillær opbygning. Disse celler påvistes i biopsier taget i forskellige afstande fra tumor. Hos en patient med vitiligo iagttoges i mange biopsier, taget omkring tumor, skrumpede melanocyter med tydelige tegn på destruktion af cellerne. Flere patienter havde melanocyter med veludviklet endoplasmatisk retikulum, Golgi apparat og forøget mængde af "lysosome residual bodies". Disse celler fandtes oftest i biopsier taget i umiddelbar nærhed af tumor.

Mest markante ændringer i hele melanocytsystemet blev iagttaget dels hos en patient med metastaserende melanom og dels hos en patient med multiple melanomer. Disse ændringer omfattede tilstedeværelse af melanosomkomplekser, granulære melanosomer, makromelanosomer, øget antal af mitochondrier og lysosome residual bodies, samt veludviklet Golgi apparatus.

Endelig fandt vi, at 8 ud af de 27 undersøgte patienter adskilte sig ved totalt fravær af ultrastrukturelle melanocytforandringer.

1.3 SEM-undersøgelse

I hudbiopsier taget tæt ved tumors makroskopiske grænse blev der i enkelte tilfælde påvist kraftigt hypertrofiske og dendritiske melanocyter med glat overflade. Desuden fandtes i få tilfælde store, runde celler med ujævn overflade besat af mikrolebs og blebs (Fig. 26). Morfologisk svarede disse celler til visse cellelinjer i primærtumor. Det kunne i øvrigt konstateres, at ovenfor nævnte celler ingen dendritisk forbindelse havde med primærtumor igennem dermo-epidermal junction.

2. Kvantitative undersøgelser

To ud af 22 patienter havde flere melanocyter omkring tumor i forhold til kontrolbiopsier fra anatomisk symmetrisk sted. Begge tumorer var klassificeret som NM. To patienter, der udviklede vitiligo omkring tumor, viste sig at have færre melanocyter i biopsierne fra de vitiliginøse hudområder. Hos 5 patienter konstateredes endvidere signifikante forskelle i melanocytantal fra flere hudbiopsier. Dette forhold havde ingen relation til afstanden fra tumor. Hos 13 ud af 22 patienter fandtes ingen

signifikante forskelle i antallet af melanocyter mellem hudbiopsier taget i forskellige afstande fra den primære tumor. I 12 ud af 22 patienter var det totale melanocytantal forøget i forhold til antallet hos normale individer.

3. Diskussion

I epidermis, i en zone fra få mm og op til 1 cm omkring såvel SSM som NM, påvist hos en del patienter såvel enkelte "suspekter melanocytformer" som maligne celler. Tilstedeværelse af enkelte maligne celler i det i øvrigt normale væv omkring malign tumor er et kendt fænomen. Således pegede Sträuli & Weiss (1977) på tre mulige mekanismer, der kan forårsage den, nemlig 1), at tumorcellerne aktivt kan vandre igennem det omkringliggende væv, 2), at tumorcellerne bliver flyttet fra tumorinfiltrat af de prolifererende normale celler, og endelig 3), at tumorcellerne er presset ind i det omkringliggende væv på grund af en trykgradient, der opstår mellem den hurtigt voksende primærtumor og det omgivende normale væv. Teoretisk set kan alle tre mekanismer gøre sig gældende i den lokale tumors invasion. På basis af mine undersøgelser er jeg af den opfattelse, at en kombination af mekanismer 1) og 2) gør sig gældende i forbindelse med den lokale invasion af melanomceller. Denne opfattelse er baseret på følgende fund: Morfologien af mikrotubuli-mikrofilamentsystem i celler, som fandtes beliggende isoleret fra tumor i det omgivende epidermis, giver indtryk af, at systemet er funktionelt labilt. Endvidere er celleoverfladen af disse celler ofte ujævn og udposet, sidstnævnte kan betragtes som et tegn på ustabilitet og aktivitet på overfladen. Sammenholdt kan disse to iagttagelser tages som udtryk for en evt. motorisk celleaktivitet.

Epidermis oven for og omkring de fleste maligne melanomer er aktiv og hypertrofisk. Jeg mener, at de hurtigt prolifererende epidermocyter også spiller en rolle ved flytning af de penetrerende maligne celler.

Hos en del patienter med SSM iagttages aktivering af de i nærheden af tumor beliggende melanocyter. Denne aktivering fandtes kun i et begrænset område af epidermis, d.v.s. få mm op til 1 cm fra tumor. Cochran (1969) er af den opfattelse, at forekomsten af aktiverede celler kan skyldes enten såkaldt "field change", som i visse tilfælde ledsager udvikling af kutant melanom, eller den repræsenterer resten af nævus, i hvilken

melanom har udviklet sig. Da jeg ikke har påvist nævusceller i områder med aktiverede melanocyter, mener jeg, at den sidstnævnte forklaring er mindre sandsynlig i mit materiale. Petersen et al. har peget på en anden mulig forklaring, nemlig at det maligne melanom er i stand til at overføre malignt potentiel til de omkringliggende melanocyter via dendritforbindelser og dermed aktivere dem. Endvidere mener Olsen, at melanocytpopulation omkring tumor bliver kontamineret af nogle af tumor udskilte substanser, der er i stand til enten at aktivere cellerne eller malignt omdanne dem. Ifølge denne teori kan kontamineringen foregå over større afstande, op til 5 cm fra tumor. Teorierne er blevet sandsynliggjort af senere kliniske iagttagelser. Seiji & Takahashi har således beskrevet melanomformer med melanocytoproliferation i den tilgrænsende epidermis. Paul & Gernand har påvist, at melanocyterne omkring visse SSM er klart større og mere dendritiske end cellerne i kontrolbiopsier.

Vores resultater blev sammenholdt med disse arbejder:

Mine morfologiske undersøgelser synes i et vist omfang at støtte kontamineringsteorierne. Men det viste sig, at melanocyttaktiveringen var begrænset til epidermisområdet beliggende i den umiddelbare nærhed af tumor. Det kunne tyde på, at det drejer sig om et lokalt fænomen. Endvidere var jeg ikke i stand til at påvise indbyrdes dendritisk kontakt mellem melanocyterne i form af dendritisk netværk og heller ikke forbindelse mellem suspekter eller aktiverede celler igennem d-e-junction. Kontamineringen må derfor nødvendigvis foregå på andre måder end ved dendritforbindelser mellem cellerne. Muligvis via penetrering af visse substanser udskilt af tumor. Enkelte af de aktiverede melanocyter var kraftigt hypertrofiske med dendritspidserne omringet af utallige melanosomer, som tilsyneladende var beliggende i det ekstracellulære rum. Det er formentlig udtryk for en funktionel forstyrrelse i den epidermale melaninenhed. Melanocyten er ikke længere underkastet enhedens fysiologiske kontrolmekanismer og producerer betydeligt flere melanosomer, end der er behov for i epidermis. Dette er muligvis det allertidligste tegn på transformering af cellen. Lерche & Maslo (1976) har påvist, at det prolifererende pigmentepitel over de chorioidale melanomer undertiden kan undergå metaplastiske forandringer. Jeg påviste imidlertid kun aktivering af melanocyter omkring SSM og slet ikke omkring NM. Undersøgelsen støtter således igen Clark-McGovern's iagttagelser om forskellig histopatologisk udvikling af

Indflydelse af melanomet på de omgivende melanocyter kan tilsyneladende vise sig på vidt forskellige måder, idet to patienter med udviklet vitiligo omkring tumor viste sig at have betydeligt færre melanocyter i de vitiliginøse områder i forhold til andre områder. Endvidere påvistes ved TEM-undersøgelser overbevisende tegn på celledestruktion hos den ene patient. Hverken i dermis eller epidermis af det vitiliginøse område blev der påvist lymfocytinfiltration. Dette tyder ikke på forekomsten af "cellemedieret" immunologisk eliminering af melanocyter i mit materiale.

I et område af normal epidermis strækkende sig fra få mm og op til 1 cm fra tumor påvistes således såvel aktivering af melanocyter som forekomst af maligne celler. Ud fra disse resultater kan anføres, at det kutane melanom ikke bør excideres i en afstand mindre end ca. 2 cm. Ved fastsættelse af denne mindste excisionsafstand skal man dog også tage andre metastaseringsmekanismer end den lokale epidermale spredning i betragtning.

Paul & Gernand har påvist, at antallet af DOPA-positive melanocyter omkring SSM er signifikant højere sammenlignet med et anatomisk symmetrisk sted. Endvidere fandt de, at melanocyttallet omkring de NM var uforandret. Imidlertid har jeg ikke kunnet bekræfte disse resultater i mit materiale. Jeg sammenlignede antal af melanocyter omkring tumor med et kontrolsted, og det viste sig, at kun 2 ud af 22 patienter havde højere melanocythyppighed omkring tumor. Begge tumorer var klassificeret som NM. Flere biopsier taget fra samme patienter viste bemærkelsesværdig stor variation i melanocyttallet. Denne variation konstateredes hos en del af patienterne. Dog var variationen uden relation til hverken tumortype eller afstanden fra tumor. Her kan Paul & Gernands resultater ikke direkte sammenlignes med mine, idet jeg har talt cellerne unuer en større forstørrelse ($\times 400$). Jeg havde derved mulighed for at iagttage også de meget svagt farvede adendritiske celler.

Sammenligning af melanocytpopulation hos normale individer (ref. III) med melanocytpopulation hos patienter med maligne melanomer (VI) viser øgning af denne hos 12 ud af 22 patienter i den sidstnævnte gruppe. Dette gav mig en mistanke om, at nogle patienter med kutane melanomer kunne have

ustabile melanocytformer i et større antal, end man kunne forvente i et normalt materiale. Der blev derfor udført en række TEM-undersøgelser på biopsier, taget fra huden omkring de maligne melanomer (VII). Ved disse undersøgelser blev der påvist melanocyter med ultrastrukturelle ændringer hos over halvdelen af patienterne. Ændringerne omfattede tilstedeværelse af: 1) kæmpemelanosomkomplekser, 2) makromelanosomer, 3) granulære melanosomer samt øget antal af "lysosome residual bodies" og bizarre mitochondria. Hos en del patienter fandtes celler med kæmpemelanosomkomplekser. Melanocyter med lignende melanosomkomplekser er også tidligere blevet påvist hos patienter med Chediak-Higashi-syndrom (Zelickson, Windhorst, White & Good 1968) og i nævus depigmentosus (Jimbow, Fitzpatrick, Szabo & Hori 1975). Fem patienter viste sig at have melanocyter med makromelanosomer. Forekomst af melanocyter med makromelanosomer blev endvidere konstateret i en række af hudens hypo- og hyperpigmenterede tilstande, såsom: X-linked ocular albinism (O'Donnel, Hambrick, Green, Iliff & Stone 1976), Xeroderma pigmentosum (Guerrier, Lutzner, Devico & Prunieras 1973), café au lait-pletter ved neurofibromatose (Takahashi 1976, Jimbow, Szabo & Fitzpatrick 1973), nævus spillus (Konrad, Wolf & Hönigsmann 1974, Eady, Sparrow & Grice 1975), lentiginosis profusa syndrome (Selmanowitz 1975) og Leopard syndrome (Bhawan, Purtilo, Riordan, Saxena & Edelstein 1976). Dersom alle nævnte tilstande har en genetisk baggrund, er det sandsynligt, at de to melanosomforandringer i mit materiale (kæmpemelanosomkomplekser og makromelanosomer) afspejler genetisk betinget forstyrrelse i melanogenesen i de enkelte melanocyter.

Ydermere skal det nævnes, at to patienter i mit materiale udviklede multiple melanomer. Hos begge patienter fandtes celler med de beskrevne melanosomanomalier og flere celler med andre ultrastrukturelle afvigelser, såsom granulære melanosomer, øget antal af lysosome residual bodies og bizarre mitochondrier. Da multiple melanomer ofte har genetisk baggrund (Wallace, Beardmore & Exton 1973, og Anderson 1972), kunne det forventes, at netop disse patienter har mange melanocytformer, hvori melanogenesen er forstyrret på genetisk basis.

På den anden side mener Kawamura, Ikeda, Mori & Obata (1966), at tilstedeværelse af melanocyter med kæmpemelanosomkomplekser i f.eks. pigmenteret Mb. Bowen afspejler en blokering af melanosomernes overførsel til de metaplastiske epidermocyter. Dannelsen af melanosomkomplekser

efter ultraviolet bestråling er sandsynligvis forårsaget af øget autofagocytotisk aktivitet i melanocyten (Zelickson, Mottaz & Hunter 1972). Endelig er der tidligere påvist melanocyter med både kæmpemelanosomkomplekser og makromelanosomer i den normale hud hos patienter med metastaserende maligne melanomer (Szekeres & Orfanos 1978, Konrad et al.; Drzewiecki, VII) og i vitiligo omkring primærtumor (Ortonne & Perrot 1978). Ifølge Szekeres & Orfanos kan malignt melanom i senere kliniske stadier ledsages af universelle forandringer i de epidermale melanocyter.

Konkluderende kan det anføres, at der forekommer flere melanocyter med ultrastrukturelle ændringer hos patienter med malignt melanom end hos normale individer. Celler med lignende ultrastrukturelle ændringer blev også fundet ved flere lidelser, hvoraf en hel del har genetisk baggrund.

Det er således sandsynligt, at de observerede ændringer i melanosomernes morfogenese hos patienter med malignt melanom er genetisk betingede.

KAPITEL VI

Resume og konklusioner

Undersøgelsen har beskæftiget sig med fire nedenstående emner:

1. Udarbejdelse af en metode, som er egnet til overflademorfologiske undersøgelser af epidermale melanocyter og maligne melanomceller in situ i hudbiopsier ved anvendelse af Scanning elektronmikroskopi (SEM), jf. kapitel II.
2. Redegørelse for de normale melanocyters morfologi, funktion og deres regionale fordeling, jf. kapitel III.
3. Komparativ undersøgelse af finstruktur samt overflademorfologi af melanomceller in situ. I forbindelse hermed blev cellemorfologien forsøgt relateret til tumortype og klinisk forløb, jf. kapitel IV.
4. Systematisk undersøgelse af de epidermale melanocyter hos patienter med malignt melanom med henblik på en vurdering af de tidligere arbejder vedrørende melanocytforandringer omkring tumor, jf. kapitel V.

De foreliggende undersøgelsesresultater kan resumeres således:

ad 1. Anvendelsen af SEM krævede en adskillelse af epidermis fra dermis. Jeg foretog denne adskillelse ved hjælp af flere forskellige metoder. En opløsning af EDTA og vævskulturmediet T-199 gav bedste resultater med hensyn til renlig adskillelse og cellepræservering. Det kunne i øvrigt konstateres, at epidermis og de tilhørende melanocyter var usædvanligt velbevarede i områder, hvor den modsvarende dermale overflade var glat. Derimod blev en del af cellerne beskadiget i forbindelse med adskillelser-

proceduren i områder af epidermis, som dækkede kraftigt udviklede dermale papiller, og hvis overflade var foldet i form af talrige cristadannelser. Sidstnævnte forhold vanskeliggjorde naturligvis en fortolkning af overflademorfologi og indbyrdes celleforhold i dermo-epidermal junction i en del af biopsierne.

ad 2. Gennemsnitligt antal og den regionale fordeling af epidermale melanocyter er tidligere blevet undersøgt af andre forskere, men på andre befolkningsgrupper. Mine undersøgelser af skandinaviske patienter bekræfter disse undersøgelser samt de tidligere fundne indbyrdes forhold mellem to celletyper i epidermis, nemlig melanocyter og epidermocyter. Således blev tilstedeværelsen af den "epidermale melaninenhed" bekræftet ved mine undersøgelser. Endvidere gav melanocyternes morfologi sammenholdt med forskellig farvbarhed med DOPA formodning om en cyklisk funktion af cellen.

ad 3. Undersøgelsen viste, at maligne melanomceller bedst kan genkendes på basis af ændringer i melanosomstruktur. Den ændrede melanosomstruktur tyder her på forandringer i cellens melanogenetiske mekanismer. Således påviste jeg tre basale melanosomformer: 1) fibrillære melanosomer, 2) granulære melanosomer og 3) vesikulære melanosomer. Det skal dog understreges, at der forekommer en overlapning mellem disse tre former i samme celle og mellem cellerne indbyrdes. Med hensyn til celledifferentiering var det kun muligt i denne undersøgelse at skelne imellem de højtdifferentierede og de lavtdifferentierede celler. Der fandtes ingen forskel i cellens finstruktur imellem SSM og NM. Men det er vigtigt at understrege, at jeg kun undersøgte den invasive nodulus af tumor. Jeg sammenholdt endvidere tumortype med det kliniske forløb og kunne derved påvise en korrelation til kernens udseende samt til mængden og morfologien af mikrofilamenterne. De maligne cellers form og overflademorfologi viste særdeles stor variation. Ligeledes observeres variation i mikrofilament-mikrotubuli systemet. En vis ensartethed blev dog påvist i følgende henseende: de runde ovale celler viste en tilbøjelighed til udvikling af ujævn overflade. En sådan overfladestruktur var i nogle tilfælde ledsaget af et bestemt mikrotubuli-mikrofilament mønster. Disse runde eller ovale celler fandtes oftest beliggende i den invasive rand af tumor, et forhold, som kunne tyde på, at de nævnte celleformer er i stand til aktivt at penetrere det omgivende væv.

ad 4. Undersøgelsen støtter Clark-McGovern-teorien om forskellig histopatologisk udvikling af SSM og NM, idet jeg kun fandt aktivering af de epidermale melanocyter omkring SSM. Undersøgelsen støtter også Petersens og Olsens aktiveringsteori, at det maligne melanom er i stand til at påvirke de omkringliggende melanocyter. Men da aktivering kun påvistes i et begrænset område af den tilgrænsende epidermis, måtte jeg i modsætning til Olsen slutte, at den omtalte aktivering var både lokal og begrænset. Forekomsten af enkelte maligne celler i normal epidermis, omkring nogle maligne melanomer, er sandsynligvis forårsaget dels ved aktiv vandring af de maligne celler og dels ved passiv flytning af dem forårsaget af kraftigt prolifererende epidermocyter. I et område af epidermis, strækken-
de sig fra få mm og op til 1 cm fra tumor, påvistes såvel aktivering af melanocyter som forekomst af maligne celler. Ud fra disse resultater kan anføres, at det kutane melanom ikke bør excideres i en afstand mindre end ca. 2 cm. Ved fastsættelsen af denne mindste excisionsafstand skal man dog også tage andre metastaseringsmekanismer end den lokale epidermale spredning i betragtning. Hos en del patienter med kutane maligne melanomer påvistes i flere biopsier beliggende fjernt fra tumor celler med ultrastrukturelle ændringer. Dette forhold kan tyde på en ændret melanogenese, som enten kan være (i) genetisk betinget, eller (ii) i mindre grad forårsaget af intercellulære påvirkninger. Disse celler er muligvis mere labile og kan lettere undergå malign transformering under indflydelse af en række eksterne faktorer. Cellerne kan dermed spille en rolle i ætiologien af det maligne melanom.

SUMMARY

The study described here has been concerned with the following four problems:

1. Development of a suitable method to examine the surface morphology of epidermal melanocytes and malignant melanoma cells in situ in skin biopsies using scanning electron microscopy (SEM) (see Chapter II).
2. A description of the morphology, function, and regional distribution of normal melanocytes (see Chapter III).
3. Comparative examination of the fine structure and surface morphology of melanoma cells in situ. In conjunction with this I have tried to relate overall cell morphology to tumour type and clinical progress (see Chapter IV).
4. Systematic examination of epidermal melanocytes in patients with malignant melanoma, in order to evaluate previously published studies on the changes seen in melanocytes surrounding a tumour (see Chapter V).

The results of these studies can be summarized as follows:

1. In order to use SEM, it was necessary to separate the epidermis from the dermis. I have achieved this using a number of different techniques. A solution of tetrasodium ethylenediamine tetraacetate dehydrate (EDTA) and tissue culture medium T-199 gave the cleanest separation and preserved cells best. It was noted that basal cells of the epidermis and the melanocytes were remarkably well preserved in areas where the corresponding dermal surface was smooth. However, damage to some of the cells in regions of epidermis covering well developed dermal papillae with a highly convoluted surface comprising numerous crista occurred during the separation process. This complicated the interpretation of surface morphology and the relationship between the various cell types in a number of the biopsy samples.
2. The mean numbers and regional distribution of epidermal melanocytes have been described by other workers for other national groups. My studies of patients of Scandinavian origin confirm these results. I was also able to detect the presence of a mutual relationship between two cell types in the epidermis: melanocytes and epidermocytes, thus confir-

ming the presence of "the epidermal melanin-unit". The morphology of the melanocytes, compared to their varying response to DOPA staining, suggested a cyclic function in these cells.

3. These studies showed that malignant melanoma cells are best recognized on the basis of changes in the structure of the melanosomes. These changes in melanosome structure suggest a different pattern of melanogenesis in malignant cells. I was able to demonstrate three basic types of melanosomes: 1) fibrillar melanosomes, 2) granular melanosomes, and 3) vesicular melanosomes. There was, however, an overlapping between these three types, both within the same cell, and from cell to cell. Therefore the degree of cell differentiation could only be classified as high or low in these studies. There were no differences in the fine structure of the cells in superficial spreading melanomas (SSM) and nodular melanomas (NM). However, it should be emphasized that only invasive nodulus of the tumour has been examined. I have also examined the relationship between fine structure of the cells, the tumour type, and the clinical progress of the disease, and was able to demonstrate a correlation to the appearance of the nucleus and to the amount and morphology of the microfilaments. The shape and surface morphology of the malignant cells showed considerable variation. There were also large differences in the appearance of the microfilament-microtubuli system. One common denominator, however, was that the round oval cells showed a tendency to develop a rough surface. This was in many cases associated with a characteristic appearance of the microfilament-microtubuli pattern. These round or oval cells were usually seen at the invasive edge of the tumour which suggests that this type of cell is able actively to penetrate the surrounding tissue.

4. The results of this study support the Clark-McGovern theory of the different histopathological development of SSM and NM, since I only found activated epidermal melanocytes around SSM. These results also support Petersen and Olsen's theory that the malignant melanoma is able to activate the surrounding melanocytes. However, I was only able to find activated melanocytes in a restricted area of the surrounding epidermis, and thus conclude in contrast to Olsen that activation is both local and limited. The presence of individual malignant cells in the epidermis in

the vicinity of a malignant melanoma is probably due both to active transplacement of the malignant cells and to passive movement caused by the rapidly proliferating epidermocytes. Both activated melanocytes and malignant cells were found in the epidermis from 1 to 10 mm from the tumour. Therefore at least 2 cm of the tissue surrounding primary tumours should be excised during primary treatment. However, other mechanisms of dissemination than local epidermal spreading should be taken into consideration when determining the minimum area to be excised. Melanocytes with ultrastructural changes were found in a number of biopsies taken at considerable distances from the primary tumour in some patients. This could suggest changes in melanogenesis that could be (i) genetic in origin or (ii) caused by intra-cellular interaction. These cells are possibly more labile, and can more readily undergo malign transformation under the influence of a series of external factors.

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ILLUSTRATIONER 

Fig. 1

Adskillelse af epidermis (e) fra dermis (d).

Fig. 2

En del af epidermis (e) er blevet adskilt fra dermis (d). Forsænkninger i epidermis (D) blev benævnt "dale"; disse svarer til de dermale papiller (P). De epidermale folder (R) blev benævnt "rygge" og svarer til forsænkninger mellem de dermale papiller (Fo).

SEM X 300.

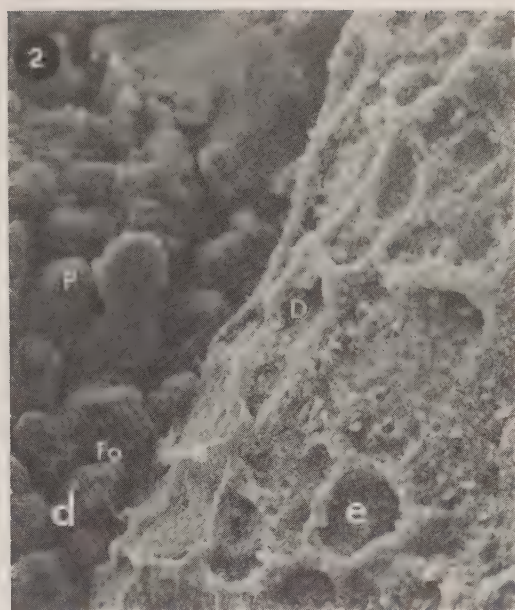


Fig. 3

Dermis med få papillae (P).

SEM X 900.

Fig. 4

Dermis. Samme biopsi som vist i fig. 3. Overfladen af dermis er forholdsvis glat med svagt udviklede cristae (C). (M) - aftryk af melanocyt.

SEM X 3.000.

Fig. 5

Epidermis fra samme biopsi som vist i fig. 3. Overfladen af såvel melanocyter (M) som epidermocyter (E) er velbevaret.

SEM X 4.000.

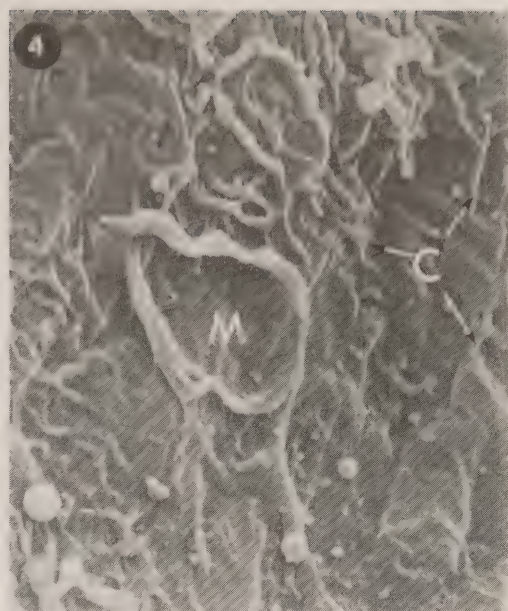
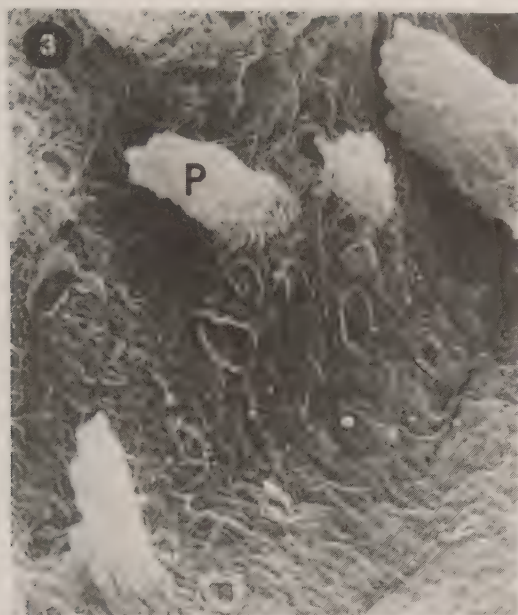


Fig. 6

Dermis med mange veludviklede papillae (P).

SEM X 100.

Fig. 7

Dermis. Samme biopsi som vist i fig. 6. Overfladen af dermis fremtræder ujævn med mange veludviklede cristae (C).

SEM X 3.000.

Fig. 8

Epidermis fra samme biopsi som vist i fig. 6. Overfladen af cellerne er dårligt bevaret, besat med utallige udposninger og blærer. Ydermere kan man se furer (F), svarende til de dermale cristae.

SEM X 10.000.

Fig. 9

Undersiden af epidermis med tilhørende melanocyter (M). (R) - epidermale rygge; (D) - epidermale dale.

SEM X 300.

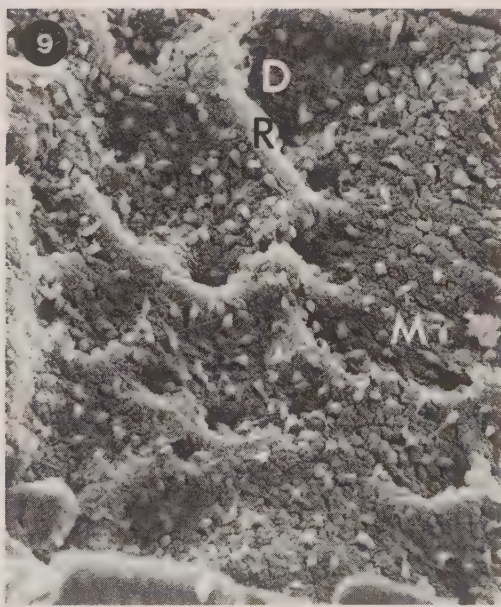
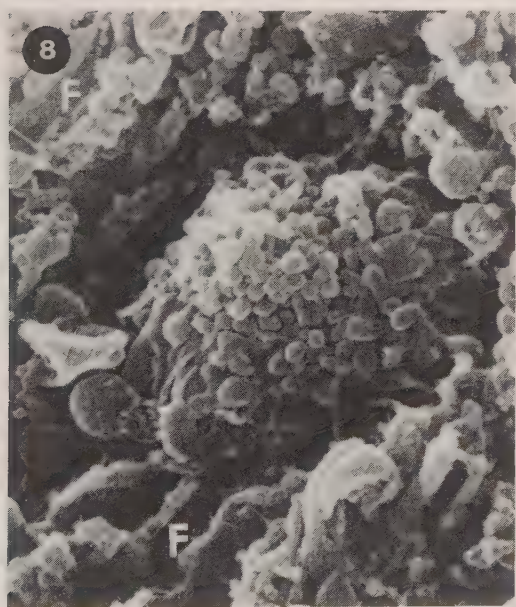
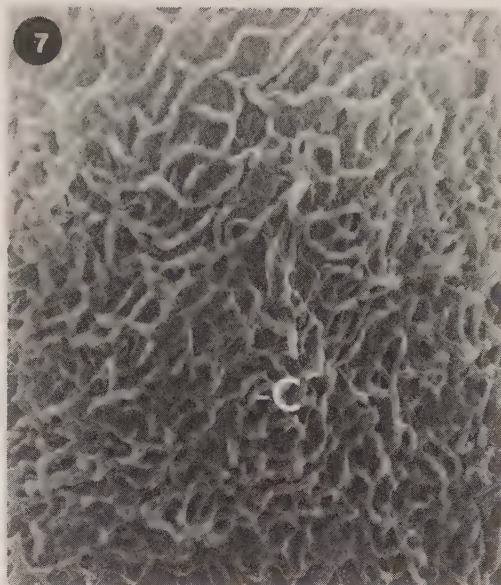
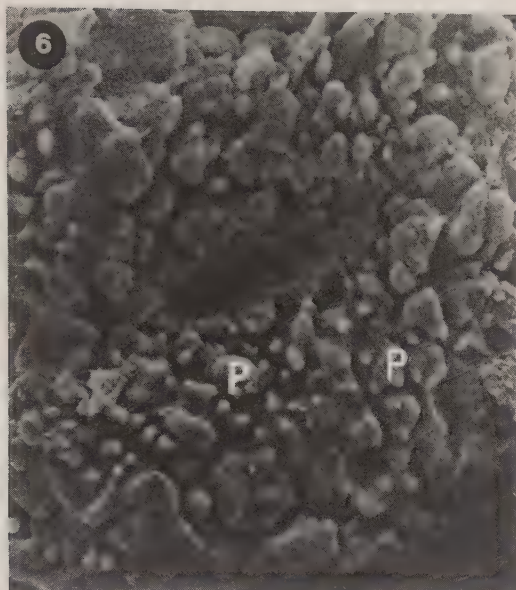


Fig. 10

Epidermis. Melanocyten (M) med dendriterne (D), som forsvinder i rummene mellem de basale epidermocyter (E). Bemærk mikrovilli (mv) på såvel melanocyten som epidermocyten.

SEM X 10.000.

Fig. 11

Epidermis. Melanocyten dendrit forløbende i dermoepidermal junction ses at afgive sidegrene.

SEM X 6.000.

Fig. 12

Epidermis. Flere dendritter (D) afgang fra melanocyterne (M); nogle af dem har fusiforme udvidelser (fu).

SEM X 5.000.

Fig. 13

Epidermis. Dendriten (D) forsvinder tilsyneladende i tragtformet formation (T) på epidermocyten overflade.

SEM X 10.000.

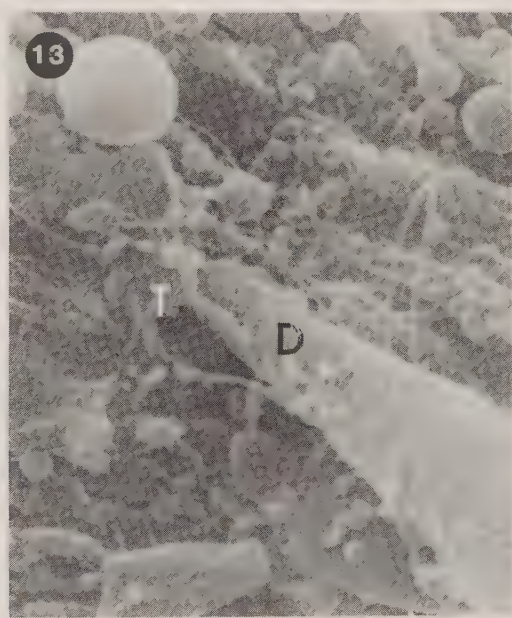
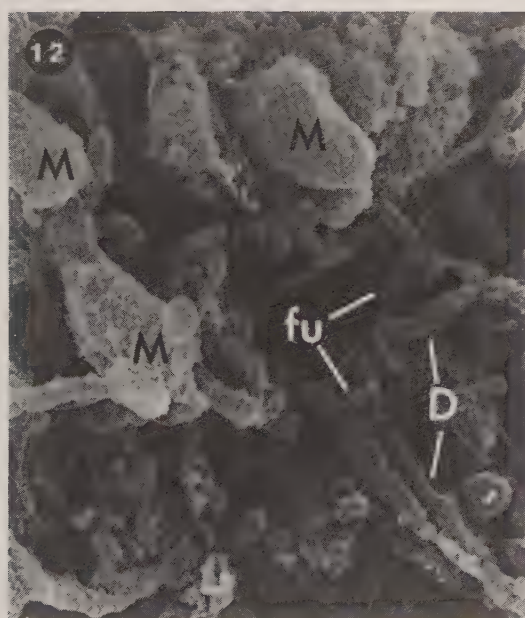
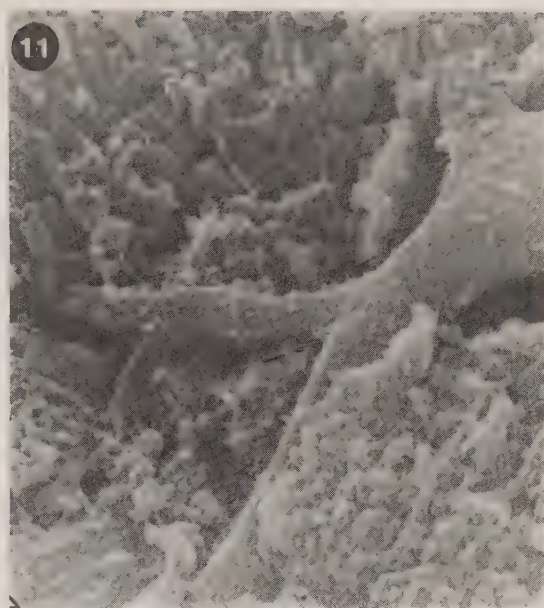


Fig. 14

Skematisk fremstilling af melanocyternes fordeling mellem de epidermale dale og rygge.

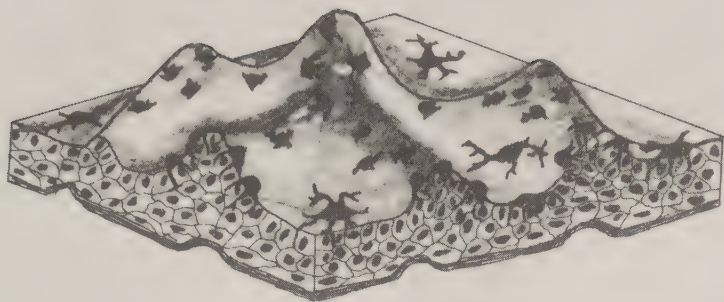


Fig. 15

Malignt melanom. Diffust infiltrerende, dendritiske celler. (K) - stratum corneum af epidermis.

SEM X 1.000.

Fig. 16

Malignt melanom. Diffust infiltrerende runde celler med ujævn overflade.

SEM X 2.000.

Fig. 17

Malignt melanom. Reder af runde, ovale celler med ujævn overflade.

SEM X 1.000.

Fig. 18

Malignt melanom. Reder af tenformede celler med glat overflade.

SEM X 600.

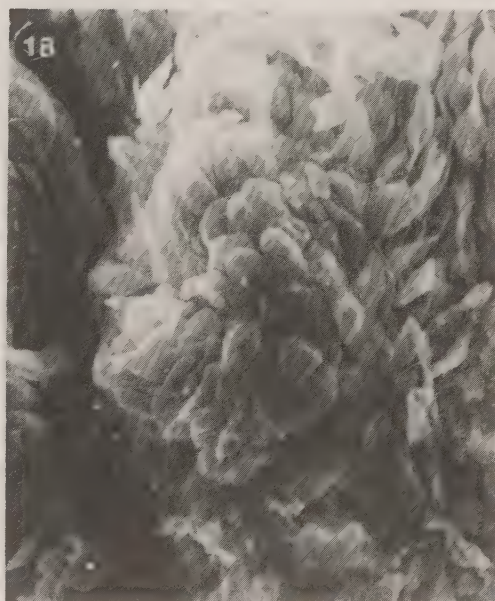
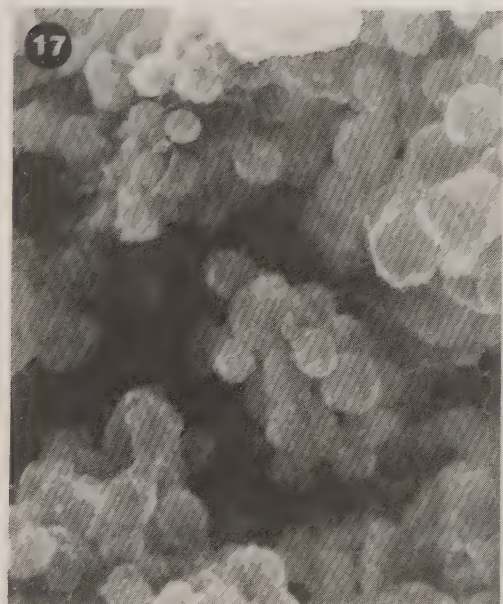
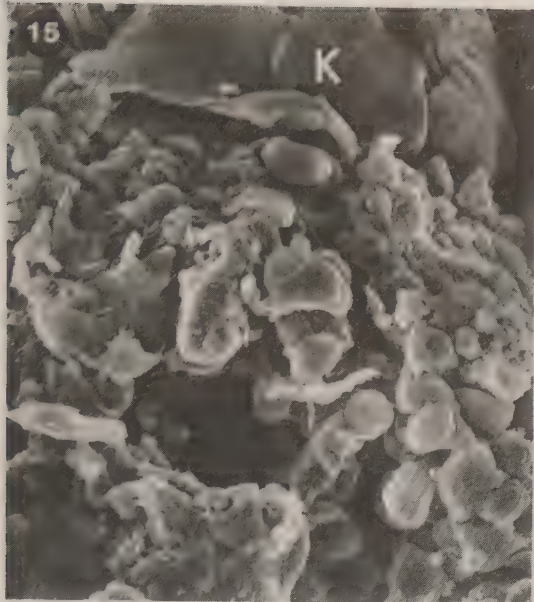


Fig. 19

Malign melanomcelle. (N) - multilobulær kerne; (Mi) - hypertrofiske mitochondrier; (mT) - mikrotubuli.

TEM X 21.300.

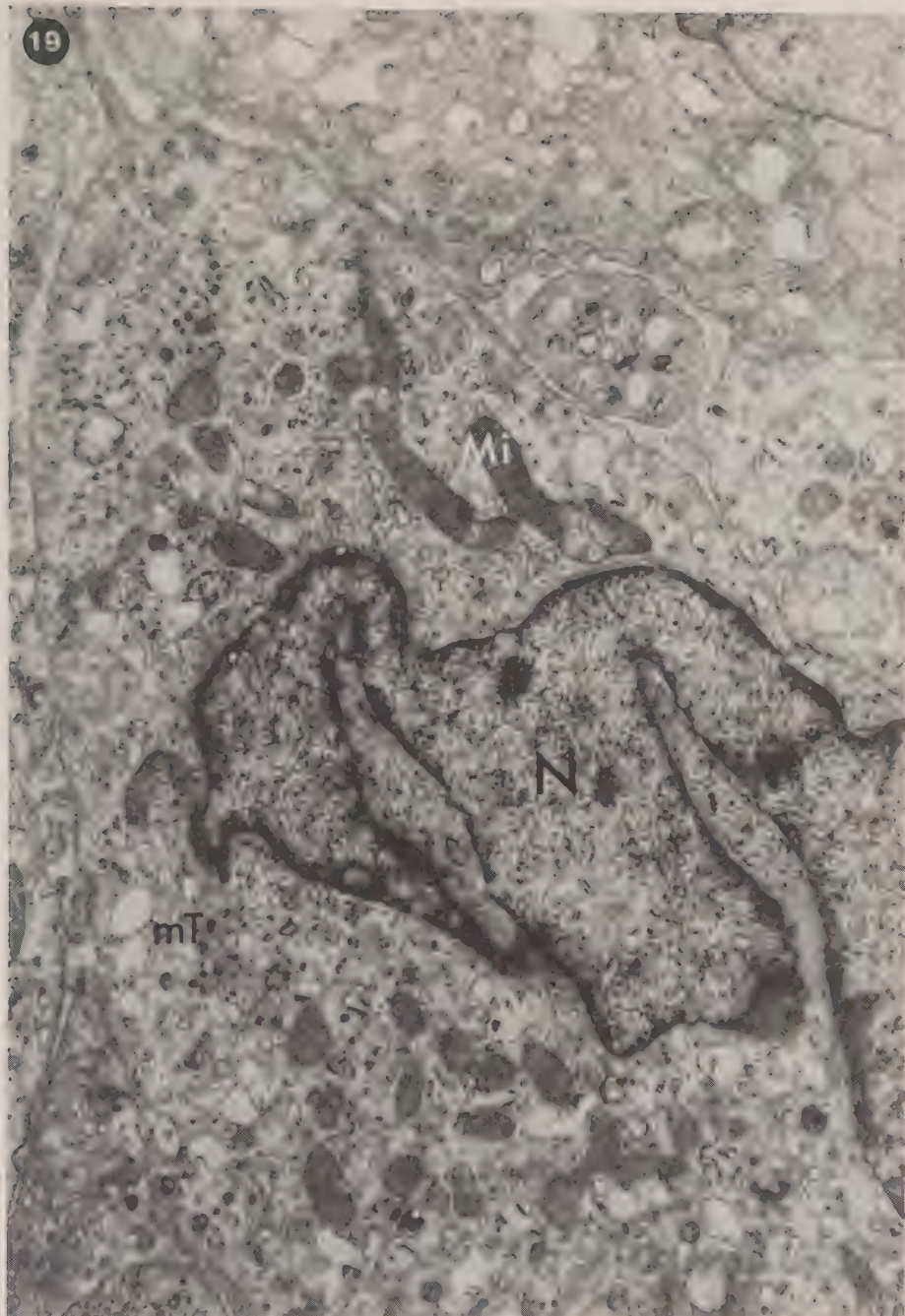


Fig. 20

Den invasive rand af malignt melanom. (D) - dermis; (M) - tre melanomceller med ujævn overflade, omgivet af zoner med fintgrynet substans (Z), hvori antallet af de kollagene fibre er væsentligt nedsat.

TEM X 12.600.

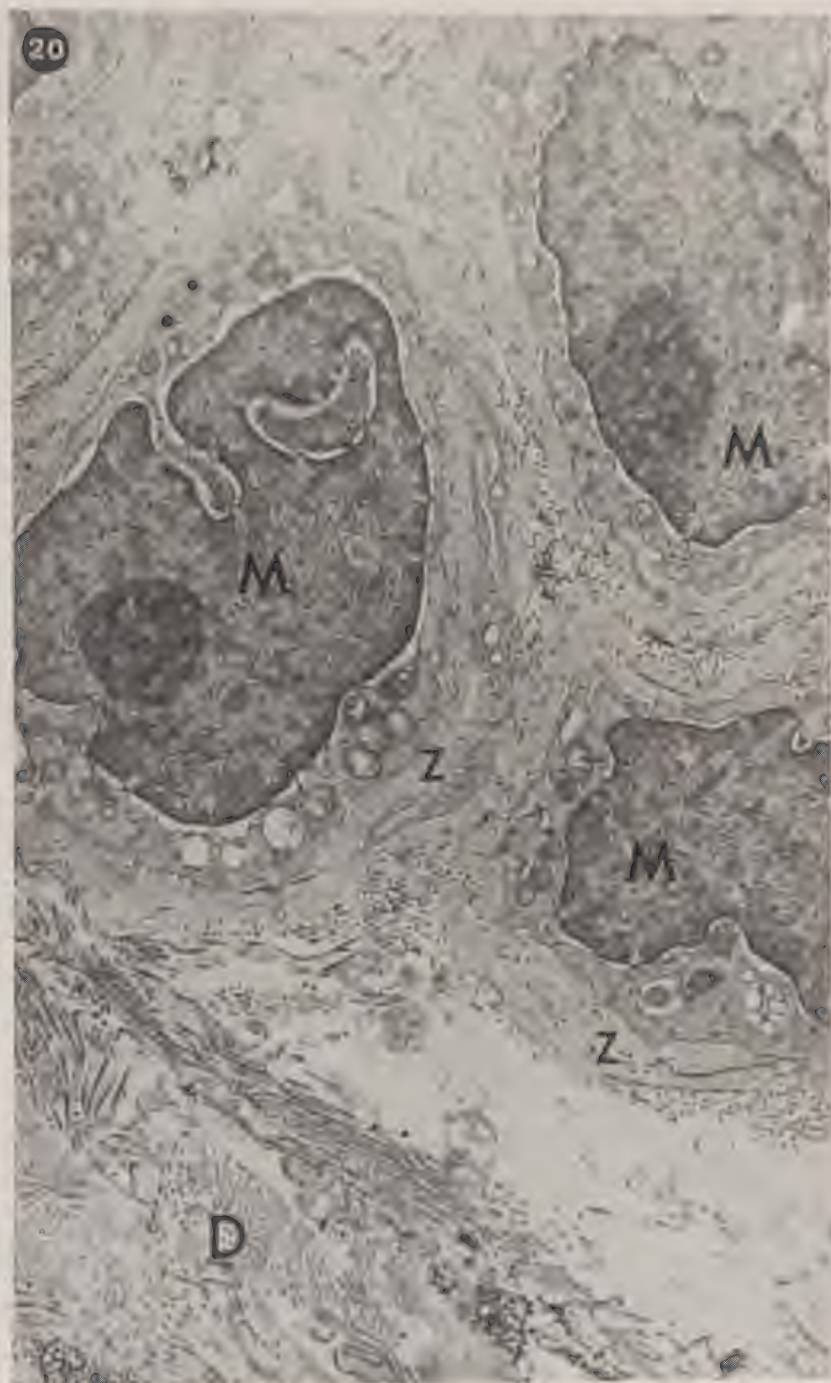


Fig. 21

Malign melanomcelle fra samme biopsi som fig. 20. (MS) - melanosomerne; (cm) - cellemembran, ujævn med dannelse af mikrovilli (mv). I cytoplasma under cellemembran ses lyse vesikler (V). Nogle af dem ses i direkte kontakt med det ekstracellulære rum (v). Cellen er omgivet af fintgrynet substans (Z), hvori antallet af de kollagene fibre er væsentligt nedsat.

TEM X 21.300.

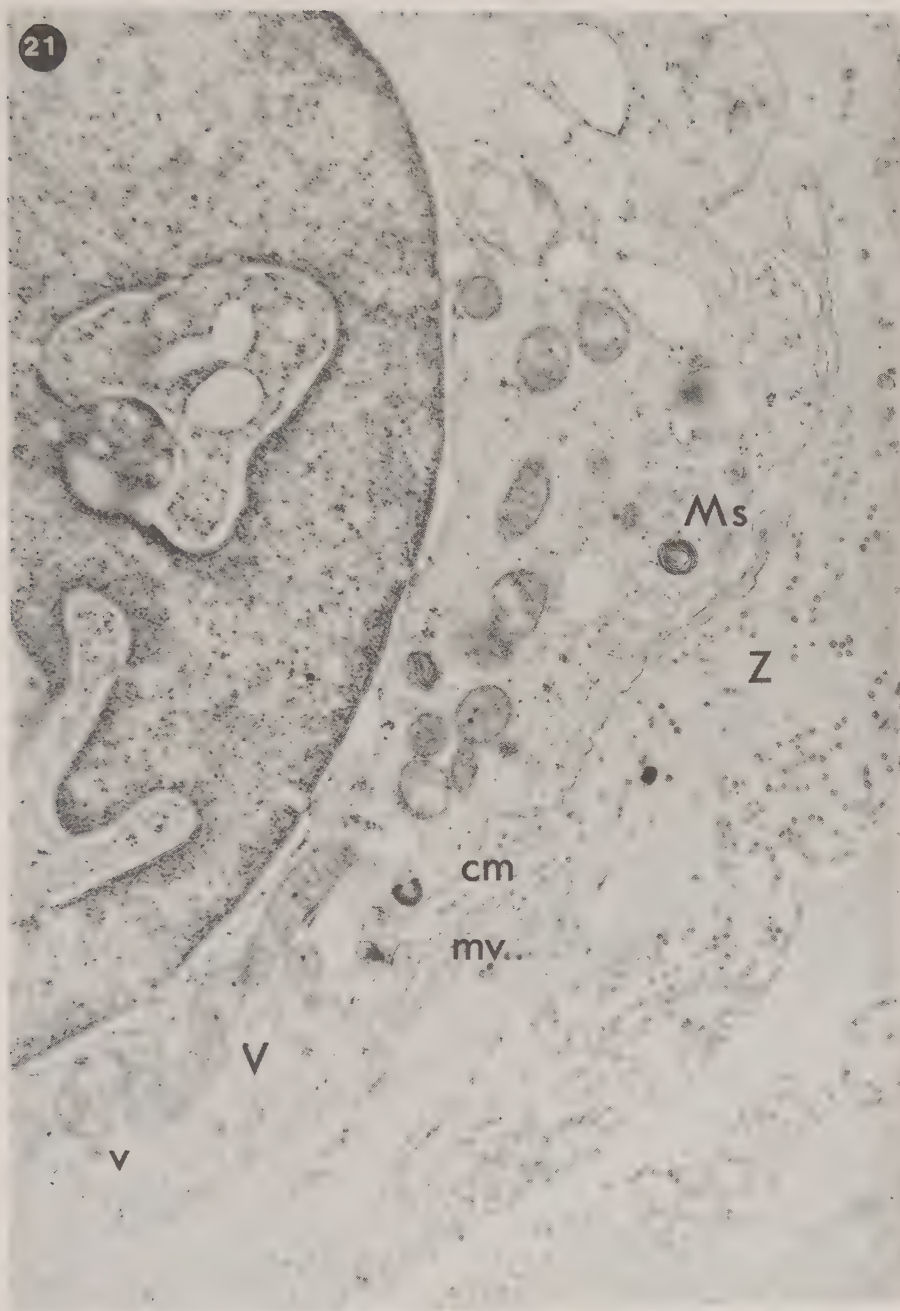


Fig. 22

Den epidermale flade farvet med DOPA. Melanocyterne er hypertrofiske, intensivt sortfarvede. Cellerne udvikler flere forgrenede dendritter.

X 800.

Fig. 23

Den epidermale flade farvet med DOPA. Melanocyterne er hypertrofiske, dendritspidserne er omringet af mange melanosomer beliggende tilsyneladende i det ekstracellulære rum.

X 800.

Fig. 24

Den epidermale flade farvet med DOPA. Cellerne er store, sortfarvede og udvikler korte, klodsede dendritter.

X 800.

Fig. 25

Den epidermale flade farvet med DOPA. Nogle af cellerne er ovale og sortfarvede. Disse celler ligner maligne melanomceller fra primærtumor.

X 800.

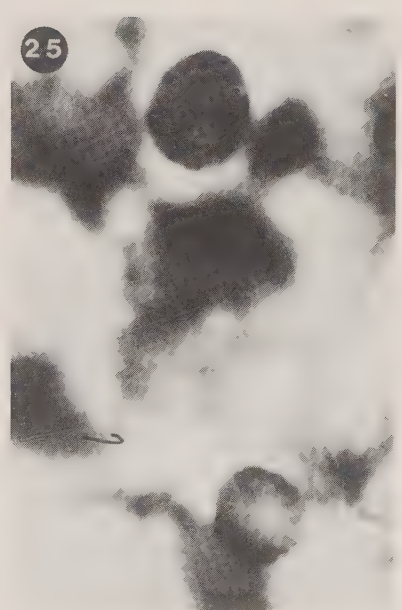
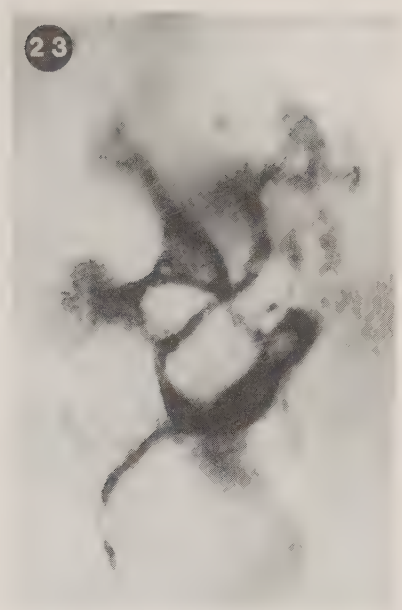
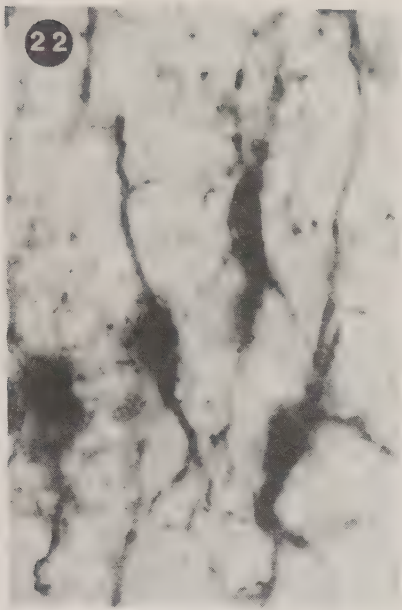


Fig. 26

Biopsi af epidermis tæt ved det kutane maligne melanom. (M) - rund celle med ujævn overflade. Denne celle ligner maligne melanomceller i primærtumor. (E) - epidermocyter.

SEM X 3.000.

26



Scanning Electron Microscopy of Melanocytes in Normal Human Epidermis

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Introduction

DOPA staining is a well-known and widely used method of studying melanocyte morphology in different regions of the human body. DOPA staining of epidermal sheets (8) has been successfully used to study the epidermal melanocyte system under varying conditions, i.e. solar irradiation (2), skin transplantation (10) and in the neighbourhood of malignant tumours (11).

DOPA staining of melanocytes in epidermal sheets from normal skin has shown (1, 6, 9) that the cell bodies form a regular pattern and that the neighbour cells anastomose via dendrites. Thus the melanocyte system appears after DOPA staining as a three-dimensional network within the epidermis. Visualization of the melanocytes in the epidermis has been based exclusively on their specific activity, the formation of melanin. In the present study an attempt has been made to visualize the unstained melanocytes in situ by means of scanning electron microscopy (SEM), by using its ability to transfer three-dimensional images of biological units.

The purpose of the present investigation was to study melanocyte density and arrangement, dendrite pattern, and cell contacts of unstained melanocytes as compared to DOPA stained cells.

Method evaluation and material

Twenty-one biopsies from the normal skin of five men and one woman were used to develop a suitable method for SEM study. The epidermis was removed from the dermis by means of sodium bromide (Table I), ethylenediamine tetraacetate (EDTA) (7), and 2.0 N sodium thiocyanate (5).

Each of the resulting sheets was divided into three pieces, which were prepared for SEM, transmission electron microscopy (TEM), and DOPA staining (8).

The specimens for SEM were washed in isotonic saline, fixed by immersion in 2.5–3.0 % glutaraldehyde (pH 7.2–7.3, 340–350 mosm) at 37°C for 1 hour, washed in 0.1 M cacodylate buffer (pH 7.2, 350 mosm), and post-fixed in 1 % osmium tetroxide for ½ hour. After another washing in cacodylate buffer for 2–4 hours they were dehydrated in increasing concentrations of acetone (30, 40, 50, 60, 70, and 90 %, 30 minutes in each, and 100 % for 3 × 10 minutes) and critical point dried using carbon dioxide. They were mounted with conductive silver paint onto brass cylinders and coated with gold and carbon in a Jeolco (type JEE-4B) ionizing vacuum evaporator, and examined in a Jeol JSM-U2 scanning electron microscope. An accelerating voltage of 16–25 kV was employed.

The TEM specimens were fixed in 2.5 % glutaraldehyde (pH 7.3) at 37°C for 1 hour, post-fixed in 1 % osmium tetroxide for ½ hour, dehydrated in increasing concentrations of ethyl alcohol and carried through propylene oxide to be embedded in Epon 812.

The DOPA specimens were prepared according to the method of Staricco & Pinkus (8).

Soaking in a 0.5 N solution of sodium bromide for 2–4 hours appears optimal for separation of the epidermis from the dermis. Lower concentration results in unclean separation. Remnants of

Table I. Separation of the epidermis from the dermis in sodium bromide solution.

Normal-ity N	Osmolarity mosm	Splitting time h	Temperature °C
0.17	317	2 – 4 – 6 – 8	37
0.50	980	2 – 4 – 6 – 8	37
1.00	>1600	2 – 4 – 6 – 8	37
1.50	>1600	2 – 4 – 6 – 8	37

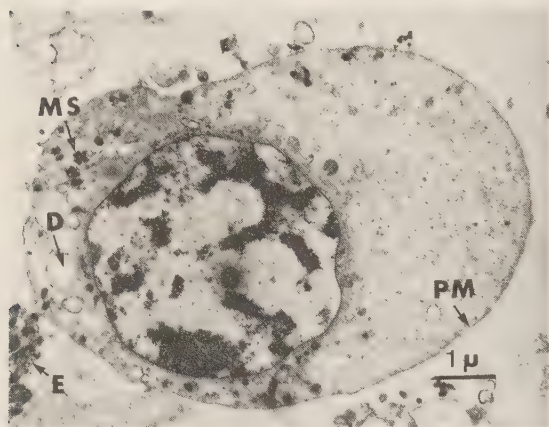


Fig. 1. Epidermal melanocyte. The plasma membrane (PM) is well preserved, cytoplasm reveals some dilated vesicles (D) and melanosomes (MS). In the left lower corner of the figure, a part of a basal epidermocyte (E) is seen. Epidermis has been separated from dermis in 0.5 N solution of sodium bromide. TEM $\times 8,000$.

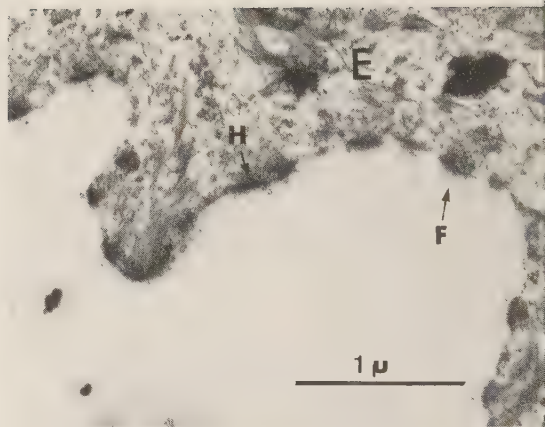


Fig. 2. The epidermal side of the dermo-epidermal junction. A basal epidermocyte (E) with hemidesmosomes (H) and filaments (F). Epidermis has been separated from dermis in 0.5 N solution of sodium bromide. TEM $\times 23,000$.

connective tissue partly cover the valeys and ridges of the dermal surface of the epidermis (Fig. 3). Higher concentrations and longer incubation time cause marked damage to many of the cells.

TEM of epidermal sheets reveals that preservation of the intracellular structures of the melanocytes is not optimal, as many mitochondria in the TEM specimens of the sodium bromide cleaved

epidermal sheets are swollen, and empty spaces can be observed in the cytoplasmic ground substance. The cell membrane, however, which is of interest in SEM study, is fairly well preserved in most of the cells (Fig. 1).

TEM of epidermal sheets separated in 0.5 N sodium bromide reveals thin filaments radiating from the hemidesmosomes of the basal epidermocytes (Fig. 2). This means that cleavage occurs



Fig. 3. A basal aspect of epidermis. Valleys (V) and ridges (R) of epidermis, remnants of connective tissue (C), and melanocytes (M) can be seen. Epidermis has been separated from dermis in 0.17 N solution of sodium bromide. SEM $\times 500$.

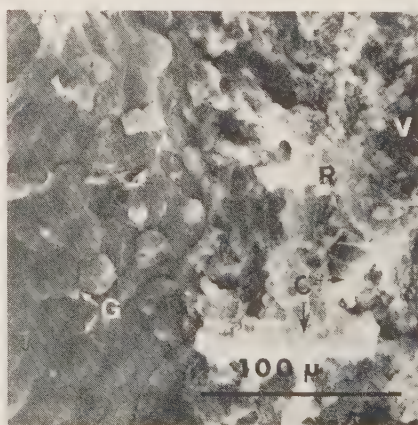


Fig. 4. A basal aspect of epidermis. Granular cells (G), remnants of connective tissue (C), valleys (V), and ridges (R) of epidermis can be seen. Epidermis has been separated from dermis in 2.0 N solution of sodium thiocyanate. SEM $\times 250$.

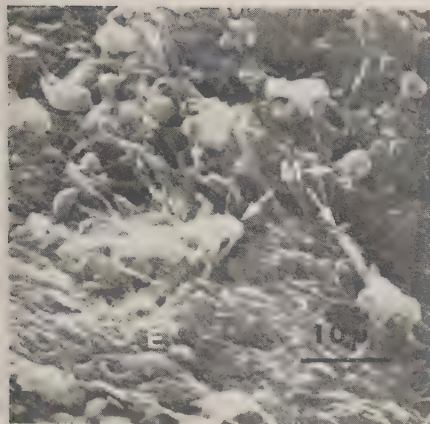


Fig. 5. Multidendritic melanocytes (M) on the dermal surface of basal epidermocytes (E). A skin biopsy from the forearm. Epidermis has been separated from dermis in 0.5 N solution of sodium bromide. SEM $\times 1,200$.

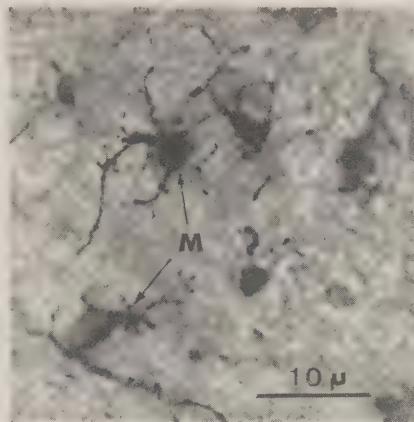


Fig. 6. Multidendritic melanocytes (M). A skin biopsy from the forearm. Epidermis has been separated from dermis in 0.5 N solution of sodium bromide, and stained with DOPA. Light microscopy $\times 1,600$.

in the same plain as that following separation with EDTA (7).

Separation in sodium thiocyanate does not occur in the dermo-epidermal junction, as is seen in SEM specimens. The bottom aspect of granular cells can be seen in some parts, in other parts the melanocytes and basal cells of the epidermis are covered by a coat of connective tissue (Fig. 4).

EDTA separation resulted in the melanocytes remaining wedge between the basal epidermocytes, so that large areas of them could not be observed. Further experiments with this solution are carried out.

Based on the above it was concluded that the optimal method of separation for the present investigation was the use of 0.5 N sodium bromide, and that the time required for such separation between 2 and 4 hours at 37°C .

Thus biopsies of normal skin from five males and two females representing nine different regions of the body were prepared using the sodium bromide technique.

Results

The density and arrangement of the melanocyte cell bodies, as seen in SEM, corresponds rather well to that seen in DOPA stained epidermal sheets (Figs 5, 6). It was not possible to detect any specific cell or dendrite pattern according to the region of the body with the exception of the specimens taken from the inner side of the

leg. In these the melanocytes were fusiform with dendrites growing into elongations of the cell bodies, bipolar melanocytes (Fig. 7).

The surface of the melanocyte cells were generally smooth with widespread smaller or larger blebs. These do not necessarily reflect biological activity. The separation procedure required treatment of the tissue in a strong hyperosmolar solution, approximately 900 mosm, for 2–4 hours prior to fixing. Thus these structures can be artifacts.

The cell bodies of the melanocytes were all situated in the dermo-epidermal junction, some were pressed down into pits between the epidermocytes, other were lying more freely and were more prominent than the cells of the epidermis.

Projections, dendrites, arise from the cell bodies of the melanocytes and run for varying distances into the dermo-epidermal junction. Some dendrites have along their course fusiform expansions (Fig. 11) and/or fungoid bud-like side shoots (Fig. 12).

The dendrites end in the dermo-epidermal junction or apparently disappear between the basal epidermocytes. Many dendrites end in the dermo-epidermal junction, either on the basal surface of the epidermocytes (Figs 7, 8) or in contact with the cell bodies or dendrites of neighbouring melanocytes (Fig. 9). The dendrite terminations were most often blunt (Fig. 8), but pointed dendrites were observed (Fig. 10). It was not possible to

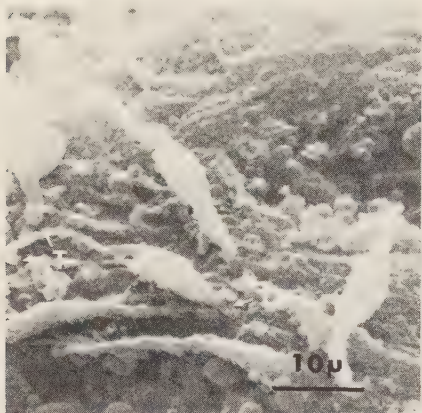


Fig. 7. Bipolar melanocytes. Some of the dendrites disappear between basal epidermocytes (D), some of them terminate blunt on their dermal surface (T). Specimen from the inner side of the leg. SEM $\times 1,200$.

All the epidermis specimens presented in fig. 7–12 have been separated from the dermis in 0.5 N_A solution of sodium bromide.

correlate the termination site of the dendrites on the epidermocytes or neighbouring melanocytes to these two forms.

Discussion

The present experience with separation solutions is that 0.5 N solution of sodium bromide gives the best results with regard to cleanliness of the splitting, cell preservation and cell presentation.

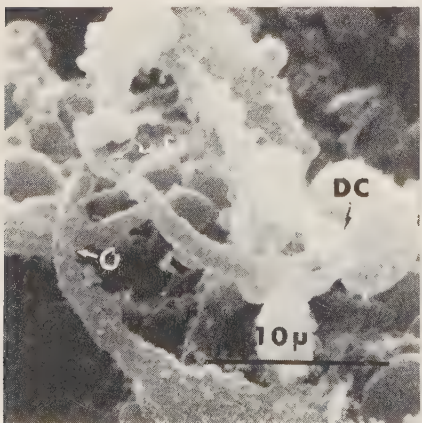


Fig. 9. Melanocytes with dendrites terminating blunt on other dendrites (D) or cell body of melanocyte (DC). Dendrites overlapping each other (O). Specimen from the inner side of the leg. SEM $\times 3,000$.



Fig. 8. Multidendritic melanocyte. Some of the dendrites disappear between basal epidermocytes (D), some of them terminate blunt on their dermal surface (T). Specimen from the forearm. SEM $\times 2,500$.

Cleavage with sodium bromide takes place through the lamina lucida in the dermo-epidermal junction. This is indicated, as extracellular portions of the hemidesmosomes are seen to be intact in TEM specimens of separated epidermis. TEM of separated epidermis reveals that many melanocytes are not optimally preserved, as some of the inner cellular structures show varying amounts of damage, such as dilatated spaces in the cyto-

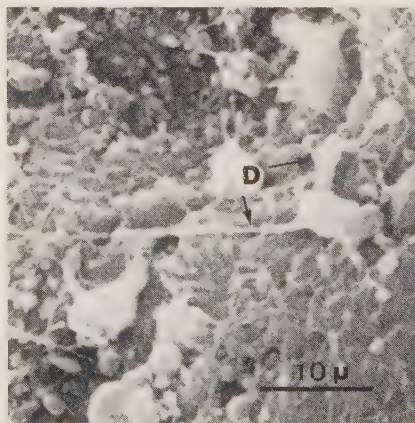


Fig. 10. Melanocyte (D) with a long pointed dendrite crossing several basal epidermocytes. Specimen from the chest. SEM $\times 1,600$.

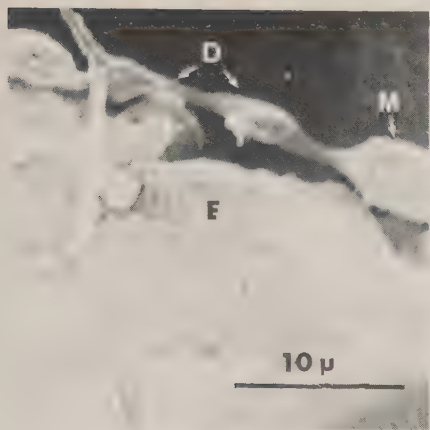


Fig. 11. Fusiform distensions of the dendrite (D). Melanocyte (M) on the ridge of rete epidermis. The dermal aspect of the basal epidermocytes (E) are shown. Specimen from the outer side of the leg. SEM $\times 2,500$.

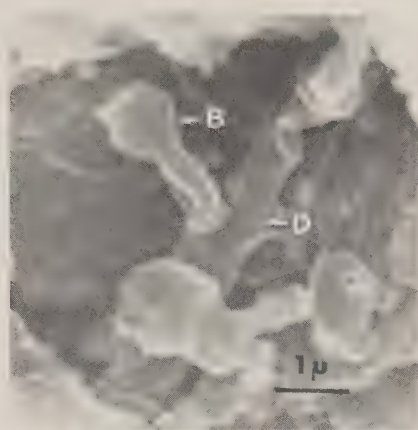


Fig. 12. Dendrite (D) with buds (B). Specimen from the face. SEM $\times 10,000$.

plasm, agglomeration of ribosomes, and swelling of the mitochondria. The cell membrane is in continuity in most of the cells.

Examination of epidermal melanocytes by SEM reveals that many of the dendrites actually run into the dermo-epidermal junction. A three-dimensional view of the specimen provides the opportunity of studying the morphology and arrangement of the dendrites. Apart from the bipolar melanocytes seen in the specimens from the inner side of the leg of the one female, most of the melanocytes are multidendritic.

Some dendrites show very distinct fusiform distentions, some of them fungoid swellings at the tip. A similar undulating pattern of dendrites is seen in phase-contrast microscopy of hamster melanoma cells growing in a medium containing compounds which activate cell function and dendrite growth (3).

Some dendrites have side buds, these are probably the place at which they begin ramification. A rare but constant observation was the presence of long thin pointed dendrites. These dendrites ran long distances in the dermo-epidermal junction, they had no buds, did not ramificate and terminated on other melanocytes or the dermal surface of basal epidermocytes.

Blunt or pointed forms of terminations ended on the basal epidermocytes or adjoining melanocytes in the dermo-epidermal junction, but termination form and site were not correlated. It is

not possible from the present study to state, whether these terminations represent specialized cell contacts or growing dendrites.

A well defined network of dendrites, as is seen in DOPA stained specimens, has not been detected with the SEM. The explanation of this may be that only dendrites running along the dermo-epidermal junction are seen in SEM, thus just a part of the epidermal melanocyte system is observed. In this dermo-epidermal part of the melanocyte system it was seen that some of the dendrites overlap. This could be the case in the upper layers of epidermis as well.

An advantage of SEM method is that the melanocytes can be studied without the necessity of histochemical staining, a process that relies on specific cell activity i.e. melanin formation. There are problems that cannot be solved by DOPA staining. For example the numerical increase of functional melanocytes following solar irradiation. It is not known, whether this is due to the activation of "dormant" melanocytes, which are invisible with DOPA staining, or due to proliferation of the melanocytes (4).

Summary

A method of examination of human epidermal melanocytes in situ by scanning electron microscopy is presented. A 0.5 N solution of sodium bromide was found to be optimal for cell pre-

servation and cleanliness of splitting. The method is compared to DOPA staining of epidermal sheet and to transmission electron microscopy. The morphology of melanocytes from nine different regions of the body has been studied. The cell bodies and the majority of dendrites of the melanocytes lay in dermo-epidermal junction, which permits their morphology to be studied from a three-dimensional aspect. Many of the dendrites had fusiform distensions, possibly an expression of sinuous movement. Some of the dendrites had buds which suggest fungoid dendrite growth.

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USE OF SCANNING ELECTRON MICROSCOPY FOR THE STUDY OF HUMAN EPIDERMAL MELANOCYTES¹

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Abstract. A method is presented which makes it possible to study melanocytes *in situ* in the human epidermis by means of scanning electron microscopy. Melanocytes are located both under and wedged between the basal epidermocytes. The dendrites describe a short course in the dermo-epidermal (d-e) junction and then ascend and disappear into the spaces between the epidermocytes. As a rule, the surface of the cells is smooth. However, proliferations such as blebs and microvilli can sometimes be observed on the surface, particularly on that of the round, adendritic cells. Contact between the melanocytes in the d-e junction is only occasionally seen.

The epidermal melanocytes are dendritic melanin-producing cells located mainly in the dermo-epidermal (d-e) junction.

Histochemical studies using DOPA have made possible the determination of the melanocyte count in different anatomical regions, as well as the description of their morphology. Both the number of melanocytes and their morphology vary considerably in the human epidermis. However, there is a certain relation between the cell form and the anatomic region. (Staricco & Pinkus, 1957). It has been demonstrated that the cell dendrites either have a horizontal course along the d-e junction or bore their way up between the epidermocytes towards the epidermal surface (Staricco & Pinkus; McGovern & Lane Brown, 1969).

Transmission electron microscope (TEM) studies have not only revealed the intracellular ultrastructure but have also indicated a relation be-

tween ultrastructure and the function of the cell (Fitzpatrick, Hori, Toda, Kinebuchi & Szabó, 1971). Recent studies indicate that the ultrastructure of the cell can change during a relatively short period of time when the skin is exposed to the influence of the sun (Jimbow, Pathak & Fitzpatrick, 1973). Hitherto, no studies have been published on the external morphology of the cell, apart from a few individual observations have been made (Moellmann, McGuire & Lerner, 1973; Wikswo, 1973). Since the cell surface is such an important part of the cell, distributing influences from adjacent cells and extracellular substances, we have deemed it desirable to investigate this point further.

The intention of the present study was to develop a method whereby Scanning Electron Microscopy (SEM) could be used to define the appearance of the melanocytes as well as the morphology of the surface of the cell *in situ* in the human epidermis. For the purpose of this *in situ* investigation it was necessary to separate the epidermis from the dermis.

In an initial study a 0.5 N NaBr solution was used for the separation (Drzewiecki & Kjærgaard, 1975). In that study it had been possible to demonstrate that both the number of melanocytes and their appearance in epidermal sheets investigated by SEM corresponded to the findings in DOPA-stained preparations. However, due to insufficient fixation it was not possible to comment on the surface morphology of the investigated cells. It was therefore necessary to improve upon the original method.

¹ Supported by grant from the Danish Anti-Cancer League.

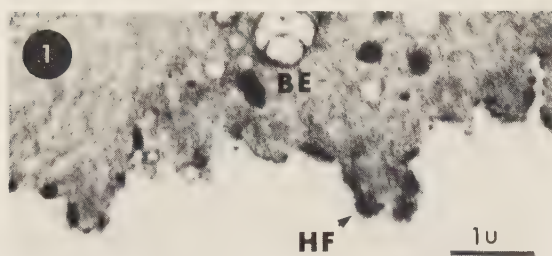


Fig. 1. TEM, $\times 10600$. Dermo-epidermal separation by use of EDTA+T199. The lowest part of a basal epidermal cell (BE) is to be seen with hemidesmosomes and their extra-cellular fibrils (HF).

METHOD

Skin from 5 patients was used. All biopsies were obtained per-operatively. The biopsies represented five anatomical regions: the breast, the upperarm, the inguinal region, the buttocks and the thigh. From each patient 2-3 biopsy specimens were excised. From 4 of the patients the biopsies were obtained from an area not exposed to the sun, whereas that from the fifth patient was obtained from a sun-affected area. All the specimens were divided for SEM, TEM and histochemical DOPA reaction studies, respectively.

For the separation of dermis from epidermis, a method

devised by Scaletta & McCallum (1967) was employed, by which the biopsy specimens were washed 2×2 min in calcium- and magnesium-free PBS at 37°C and then incubated in a solution of EDTA+PBS for 2-3 hours at a temperature of 37°C . The method has been modified inasmuch as a solution of EDTA+T199, also for 2-3 hours at 37°C , has been used instead of the EDTA+PBS. In a few biopsies 0.5% trypsin was added as well. The epidermis was separated from the dermis without much trouble, though separation was easiest with biopsies incubated in EDTA+T199. The epidermal sheets thus released were divided and prepared for SEM and TEM studies, respectively.

SEM preparation

The epidermal sheets were fixed in 2.5-3% glutaraldehyde (pH 7.2; 314 mOsm) for 1 hour at 37°C then washed in cacodylate buffer at room temperature, post-fixed for $\frac{1}{2}$ hour in OsO_4 , also at room temperature, and then transferred to cacodylate buffer and stored at 4°C . The preparations were subsequently dehydrated in ascending concentrations of acetone 30, 40, 50, 60, 90 for 15 mins each, and finally 20 min in 100% acetone. Critical point drying was carried out with carbon dioxide and the preparations were mounted on brass cylinders with the aid of conductive silver paint. The preparations were coated with gold and carbon in a SEM coating unit (E 5000 Polaron Equipment Ltd.). A Jeol JSM-U2 scanning electron microscope with an accelerating voltage of 16-20 kV was used for the studies.

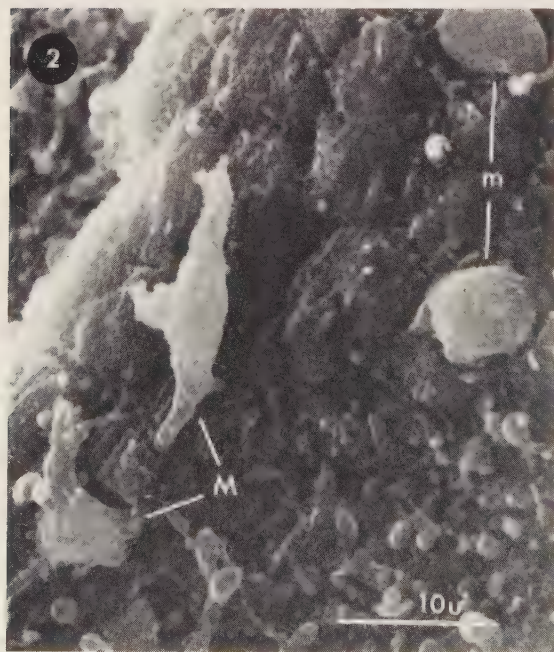


Fig. 2. SEM, $\times 2070$. Dermal surface of epidermis separated from dermis in EDTA + T199 solution. Oblong, dendritic melanocytes (M) are seen side by side with more rounded melanocytes (m).



Fig. 3. SEM, $\times 2070$. Dermal surface of epidermis separated from dermis in EDTA+T199 solution. Oval undulating dendritic melanocytes (M) dominate.

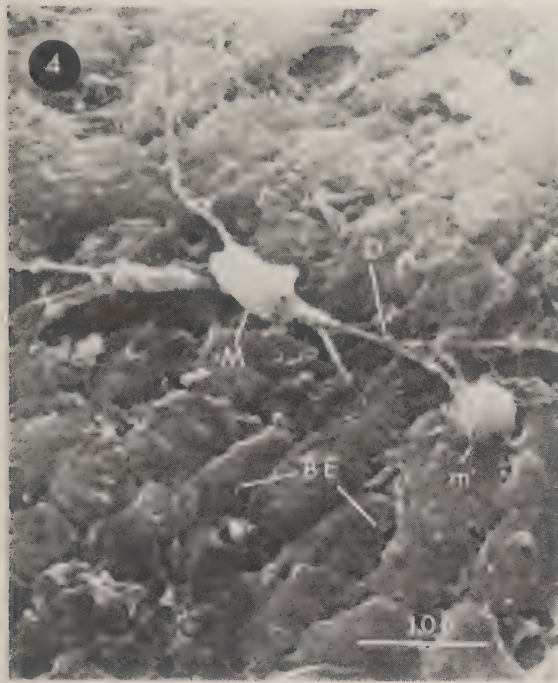


Fig. 4. SEM, $\times 2000$. Dermal surface of epidermis separated from dermis in EDTA+PBS solution. Two melanocytes (*M*), (*m*) are in contact via thin, pointed dendrite (*D*). The dermal surface of the basal epidermocytes (*BE*) can be seen clearly.

TEM-preparation

The epidermal sheets were washed in PBS, 2×2 min, fixed for 4 hours in 2.5–3% glutaraldehyde at 37°C , washed in cacodylate buffer for 1 hour at room temperature and post-fixed for 1 hour in OsO_4 at room temperature. The preparations were transferred to cacodylate buffer and stored at 4°C . Dehydration was then undertaken in ascending concentrations of ethyl alcohol and the samples were carried through propylene oxide to embed in Epon 812. The electron microscope employed was an Hitachi model HS-8.

Histochemical DOPA reaction was carried out according to the Staricco & Pinkus method with one minor modification. In place of the 2 N solution of NaBr, a 0.5 N solution was used.

RESULTS

Both EDTA+T199 and EDTA+PBS solutions are able to separate epidermis from dermis through the dermo-epidermal (d-e) junction. The separation takes place through lamina lucida for by TEM study hemidesmosome fibrils located extra-cellularly can be demonstrated (Fig. 1). The addition of 0.5% trypsin to the two solutions mentioned above

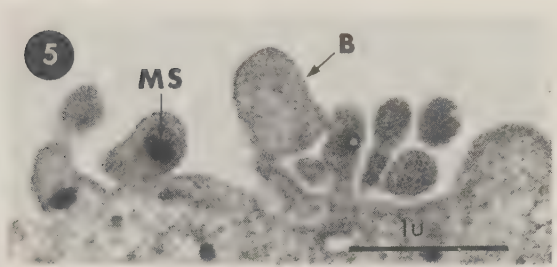


Fig. 5. TEM, $\times 25\,200$. Surface of melanocyte with bleb formations (*B*). In one of these a melanosome (*MS*) can be seen.

caused a separation through an unspecified layer of epidermis, often above the basal epidermocytes. Moreover the cells proved to be poorly fixed.

After altering the separation technique, as in the initial study (Drzewiecki & Kjærgaard), it could be ascertained that both the distribution of the melanocytes as well as their appearance in the SEM studies corresponded to the findings in the studies with DOPA-stained epidermal sheets, and that the method elaborated above should, therefore, be regarded as well suited for the purpose.

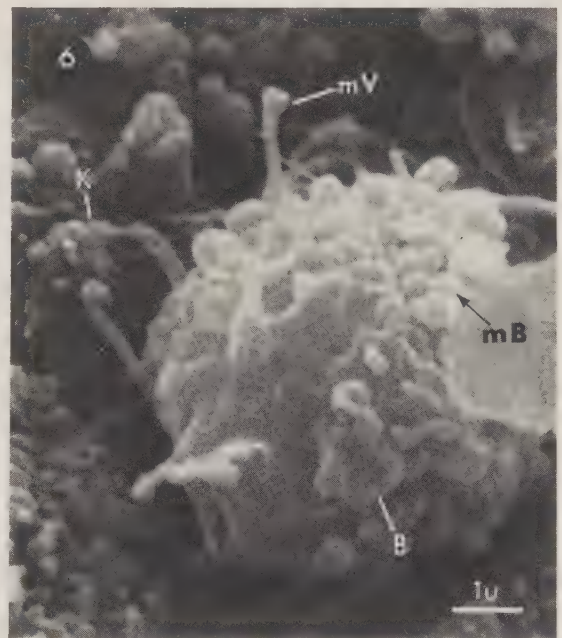


Fig. 6. SEM, $\times 11\,200$. Dermal surface of epidermis separated from dermis in EDTA + T199 solution. Numerous proliferations such as microvilli (*mV*) microblebs (*mB*) and blebs (*B*) are to be seen on the surface of round adendritic melanocyte. Some of the microvilli are in contact (*K*) with basal epidermocytes.



Fig. 7. SEM, $\times 8000$. Dermal surface of epidermis separated from epidermis in EDTA + T199 solution. Thick dendrite (D) projects from the melanocyte (M), ascends and disappears into the spaces between the basal epidermocytes (BE).

The morphology of the melanocytes is extremely varying and in the same preparation it is possible to observe elongated dendritic cells, oval and spherical cells (Fig. 2). However, in skin biopsies from areas that have been exposed to sunlight, multidendritic cells prevail, while, on the other hand, in the non-exposed areas, rounded uni-dendritic cells are seen (Fig. 3).

The study reveals that there are two types of dendrites—a point which does not seem to have been demonstrated before—a thick type, and a slender, elongated one.

The thick dendrites, which as a rule after a short course disappear between basal epidermocytes (Fig. 7), possess the ability to project secondary branches that are also relatively thick, particularly in biopsies from areas exposed to the sun. Some of the dendrites have fusiformed enlargements along their course (Fig. 8).

The slender dendrites are located mainly in the d-e junction. A number of these can be observed in contact with other melanocytes (Fig. 4).

It is not possible to demonstrate a uniform contact pattern between basal epidermocytes and the

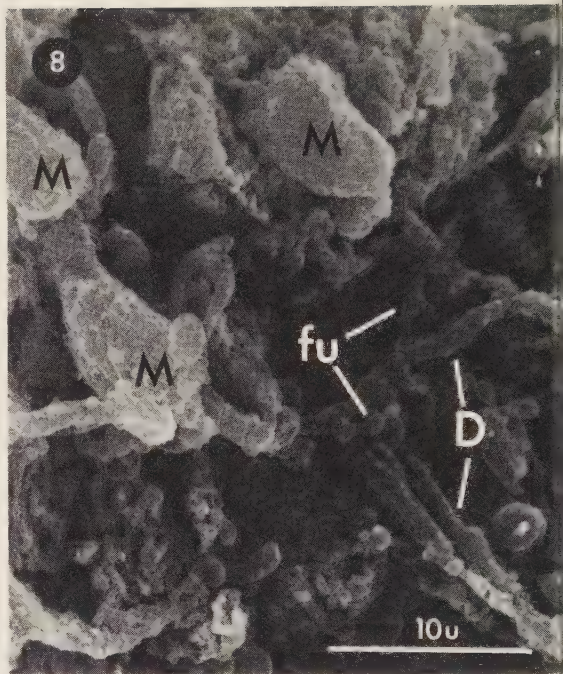


Fig. 8. SEM, $\times 5000$. Dermal surface of epidermis separated from dermis in EDTA + PBS solution. Numerous dendrites (D) proliferate from the melanocyte (M). Some of them have fusiformed enlargements (fu).

melanocytic dendrites. A particular point of interest is the observation that the thick dendrite possesses the ability to invade through opening in the cell membrane into the basal epidermocyte (Fig. 9). In a few preparations it is possible to observe that melanocytes that are in mutual dendritic connection have differing surface morphologies, so that one of the cells demonstrates numerous bulges—the so-called bleb formation—in contrast to the other cell which has a completely smooth surface.

In most cases the surface of the melanocyte is fairly smooth. But, as already described, there are cells that present numerous surface bulges and proliferations such as blebs, microblebs and microvilli. The surface of the round adendritic melanocyte is covered with more microblebs and microvilli than that of the elongated dendritic cells (Fig. 6).

Microvilli project either from the smooth surface or from the top of microblebs. Their diameter is $0.1\text{--}0.2\text{ }\mu\text{m}$ and their length is variable. They can be observed as small, twisting formations on the cell surface but can also appear stretched, in contact with the basal epidermocytes. As a rule they have a bulb-like terminal swelling.

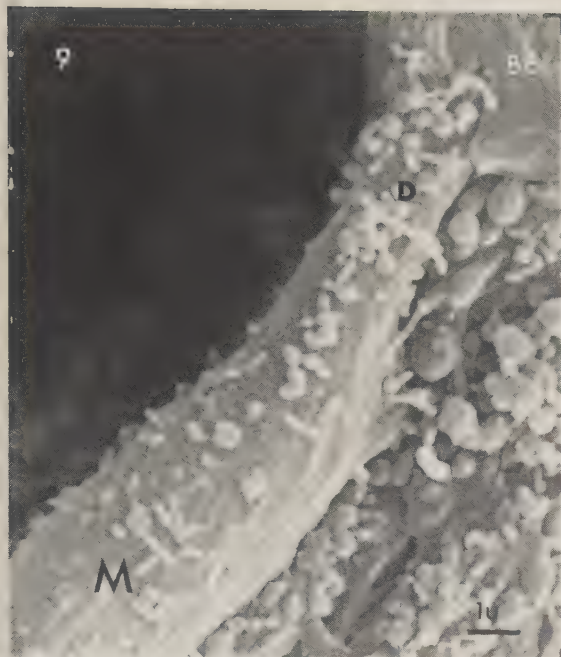


Fig. 9. SEM, $\times 9000$. Dermal surface of epidermis separated from dermis in EDTA+T199 solution. An oblong melanocyte (M) is to be seen with its dendrite (D) invading the basal epidermocyte (BE) through an opening in its surface.

Blebs appear as almost spherical formations on the surface of the cell. They have varying diameters, from $0.5\text{--}0.7\text{ }\mu\text{m}$. TEM studies reveal that they contain either an amorphous substance or melanosomes (Fig. 5).

DISCUSSION

The use of EDTA+T199 and EDTA+PBS for the separation of epidermis from dermis makes SEM studies of the surface of melanocytes possible. A solution of EDTA+T199 is, however, best suited for the studies undertaken, both as far as the practical side of the separation is concerned and also for the subsequent cell fixation. However, it should be stressed that a number of cells showed mechanical damage arising from the separation. These cells are easily recognised in SEM.

In the investigated epidermal sheets, occasional spaces in the preparations were observed corresponding to dropped-out melanocytes. These cells were rediscovered lying in hollows between the papillae on the dermal surface of the skin. This finding could possibly explain the differing figures

published of the melanocyte count per square millimetre of skin (Szabó, 1954; Staricco & Pinkus).

The morphology of the cells varies as far as shape, size and number of dendrites are concerned. No correlation was found in the present material between the cell morphology and the anatomical region. On the other hand, the morphology seems to be dependent on the activity level of the cell. Increased melanin production, intracellular transport and the transference of melanin to the epidermocytes is accompanied by dendritic proliferation as the number, size and development of secondary branches increases.

Two types of dendrite could be observed: one being thick, and the other, slender and more pointed.

As a rule, the thick dendrites have a quite short course through the d-e junction, then turn upwards and disappear into the intracellular spaces between the basal epidermocytes (Fig. 7). Often, particularly in sun-exposed areas, they have fusiform expansions along their course (Fig. 8). TEM observations reveal that these dendrites contain not only melanosomes but also microtubuli and microfilaments, which shows that they are used to transport and transfer melanosomes to epidermocytes (Jimbow et al.; Magun, 1973; Moellmann et al.). The fusiform expansions described above might be regarded as a growth pattern, but could just as likely reflect an active transport of melanosomes.

In the present study only a few contacts have been found between the melanocyte dendrites and the basal epidermocytes. This condition can partly be explained by the fact that the method employed here only permits observation of the dermal side of the epidermis and the melanocytes associated with this. It is probable that most of the cell contacts occur above the dermo-epidermal junction. The few observed contacts between melanocyte dendrites and the epidermocytes do not reveal any special pattern. In a very few cases invasion of dendrites has been observed in the basal epidermocytes, possibly with the intention of transferring melanosomes (Mottaz & Zelickson, 1967; Moellman et al.; Wikswo; Wolff, 1973; Wolff, Jimbow & Fitzpatrick, 1974).

The slender, pointed dendrites are seen to describe a longer course through the d-e junction then either to disappear up between the basal epidermocytes or to contact other melanocytes (Fig. 4). Here too it is impossible to discern the presence of a

special contact pattern. In isolated cases it can be observed that one of the cells in a melanocyte pair in mutual dendritic contact develops numerous bulges on its surface. It could therefore be assumed that information is passed between the melanocytes (Petersen, Bodenham & Lloyd, 1962) via these dendrites.

As a rule, the surface of the melanocyte is smooth, but some of the cells present innumerable bulges and proliferations such as blebs, microblebs and microvilli. The significance of these structures is not known. There is no definite correlation between the form of the cell and the presence of these structures. However, the rounded adendritic cell forms appear to have more of these structures than the dendritic cell forms. Microvilli will increase the surface of the cell to a considerable degree (Follett & Goldman, 1970) and their presence is probably an expression of the ability of the cell to maintain its surface area when the cell is in an adendritic or oligodendritic phase. By TEM studies it can be revealed that a number of blebs contain melanosomes. Thus, it is probable that the formation of some blebs represents the incipient stage of the dendrite. Microblebs appear to be an initial stage for microvilli in that they appear together in large numbers on the same cell surface. Microvilli are 0.1 and 0.2 μm in diameter; their length varies. Microvilli have not been studied by TEM as it has not been possible to demonstrate them with sufficient certainty in the preparations studied so far.

SEM studies of the preparations show that many microvilli are in contact with adjacent epidermocytes. Additionally, it can be observed that at the point of contact they develop a bottle-shaped expansion. The significance and nature of these contacts are unknown, but it could be possible to take them as evidence for an epidermal melanin unit (Fitzpatrick et al.) consisting of a melanocyte and a certain number of epidermocytes that are in mutual functional contact.

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THE EPIDERMAL MELANOCYTE SYSTEM IN INDIVIDUALS OF SCANDINAVIAN ORIGIN, DETERMINED BY DOPA-STAINING AND TEM¹

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(Submitted for publication March 20, 1979)

Abstract. The quantitative evaluation of DOPA-positive epidermal melanocytes in 16 patients of Scandinavian origin showed both individual and regional differences in the melanocyte count. Our data is in agreement with other published studies. The distribution in the number of melanocytes varies significantly in some specimens. This is due partly to the preparation procedure and partly to normal biological variations. We believe that we have demonstrated a cyclic function of the melanocyte in the epidermis. The varying density of cells in epidermal sheets as well as their varying morphology support the theory concerning the presence of the epidermal melanin unit.

In an earlier study (Drzewiecki, 1979) quantitative and qualitative determinations were made of the epidermal melanocyte system in patients with cutaneous malignant melanomas. This study showed that the melanocytes surrounding the tumour were in some cases activated. Activation occurred in a zone from a few mm up to 1 cm from the tumour. Additionally, it was demonstrated that the total melanocyte population was increased in some patients with malignant melanomas. This conclusion was exclusively based on a comparison between the melanocyte population in our material and those in other studies (Staricco & Pinkus, 1957; Mitchell, 1963; Szabo, 1967a). The methods of counting (counting field and magnification) differed mutually among the quoted authors as well as from that used in our study. Therefore, we have judged it as necessary to obtain a control material from the same population in which we employed the same methods as used in the earlier study. In addition to the quantitative determinations, we also desired to investigate the morphology of the melanocytes as well as the function of the epidermal melanin unit (Fitz-

patrick & Breathnach, 1963; Fitzpatrick, Hori, Toda, Kinebuchi & Szabo, 1971) on a material of patients without pigment disturbances by using DOPA-staining and transmission electron microscopy (TEM).

MATERIALS AND METHODS

16 patients of Scandinavian origin, aged from 4 to 89 years, with a mean age of 46 years were studied. There were 7 women and 9 men. The patients were treated for various diseases (Table I). Skin specimens (1.5×0.5 cm) were obtained during operation at a good distance from the operation field. From two patients Nos. 1, 4, two biopsies were taken from the front of the trunk and back of the trunk respectively; in the remainder only one specimen from each patient was taken. The epidermal melanocytes has been investigated in the following four anatomical regions: front of the trunk, back of the trunk, face and lower leg.

The skin specimens were divided into four parts. One was prepared for Transmission Electron Microscopy (TEM) (Drzewiecki & Kjærgaard, 1977). The other three parts were incubated in 1.0–2.0 N solution of NaBr for approximately 3 h. Afterwards the epidermis was separated from the dermis under dissection microscope. The separated epidermal sheets were incubated with DOPA (Staricco & Pinkus). All three epidermal sheets from each patient were used for quantitative and qualitative evaluation of the melanocytes. A total of 54 epidermal sheets were investigated from the 16 patients. The melanocytes were counted in 5–10 areas, chosen at random, each 0.1089 mm² using a graticule fitted in the eyepiece of the microscope, and at a magnification of ×400. The melanocyte count was expressed as the number of cells per 1 mm² of an epidermal surface. One-way analysis of variance was employed to determine the inter-regional and intra-regional (individual) difference in the melanocyte count.

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Table 1. *Distribution of material according to anatomical region for biopsy. Results of melanocyte count*

Anatomical region	Patient No.	Sex	Age	Disease	Mean ($\pm$ S.E.) of melanocyte count	Mean ($\pm$ S.E.) for region
Front of trunk	1	F	54	Breast hypertrophy	761 ($\pm$ 24)	996 ($\pm$ 34)
	2	M	58	Urethral stricture	844 ($\pm$ 41)	
	3	M	6	Hypospadia	1 397 ($\pm$ 69)	
	4	F	46	Breast hypertrophy	1 116 ($\pm$ 61)	
	5	M	78	Urethral stricture	842 ($\pm$ 47)	
Back of trunk	1	F	54	Breast hypertrophy	912 ($\pm$ 73)	1 278 ($\pm$ 76)
	6	F	30	Breast hypertrophy	1 770 ($\pm$ 77)	
	4	F	46	Breast hypertrophy	989 ($\pm$ 70)	
Face	7	M	39	Basalioma of the nose	3 245 ($\pm$ 168)	2 286 ($\pm$ 109)
	8	F	36	Seborrheic verruca	2 853 ($\pm$ 181)	
	9	M	84	Basalioma of the ear	1 793 ($\pm$ 121)	
	10	M	89	Basalioma of the ear	1 433 ($\pm$ 91)	
	11	M	4	Fibroma of the forehead	1 475 ($\pm$ 68)	
Lower leg	12	F	77	Leg ulcer	773 ($\pm$ 55)	1 564 ($\pm$ 82)
	13	F	76	Leg ulcer	1 070 ($\pm$ 40)	
	14	M	53	Nasal fracture	2 455 ($\pm$ 126)	
	15	M	22	Burn wound of the leg	1 488 ($\pm$ 103)	
	16	F	36	Verruca of the cheek	2 055 ($\pm$ 92)	

RESULTS

Quantitative determination of the melanocyte population

In the epidermal sheets with oligo-dendritical melanocytes, areas were found where melanocytes had been torn out during the separation procedure (Fig. 1). In some epidermal sheets the number of melanocytes was irregularly distributed. Moreover, in most cases a greater number of melanocytes were found around the epidermal ridges than in the valleys. In the epidermal sheets from the front of trunk, back of trunk, face, and lower leg were found 996, 1 278, 2 286 and 1 564 cells/mm², respectively (Table I). There were significant differences ($p < 0.01$) both between the patients within the investigated regions and between regions. In 5 patients, Nos. 1, 2, 8, 9, 14, significant differences were found between the quantity of melanocytes in the individual epithelial specimens of the same patient ($p < 0.05$).

Qualitative determination of the melanocyte population

No relationship was found between the morphology of the melanocytes and the anatomical region. The melanocytes on the ridge of the epidermis are often oval and oligodendritical, whereas those melano-

cytes in the valleys are, as a rule, larger, more polygonal than those on the ridges and develop a larger number of branched dendrites. In dense melanocyte populations uniform, small and round multidendritical cells, as a rule, are seen. In sparse populations, however, a generally pronounced polymorphy of the melanocytes is observed. The varying morphology and alternating intensity of the DOPA-staining lead to the suspicion of a cyclic melanocyte function. It is to be presumed that the cycle commences with a weakly coloured, oval adendritic cell. An increasing black colouring accompanies the development of more dendrites. At this point the melanocyte can best be seen for the light epidermocytes form a good, strongly contrasting background. As a result of the transference of melanin granules from the melanocyte to the surrounding epidermocytes, these are strongly brown coloured. After this activity the melanocyte shrinks, fades and finally can no longer be discerned, overshadowed by the intensely brown coloured epidermocytes (Fig. 2). In areas with weaker coloured epidermocytes it is possible, now and then, to discern the shrunken, faded melanocytes (Fig. 3). In some epidermal sheets we have observed that the above-mentioned alterations include several melanocytes at the same time in a well-defined area of the epidermis (Fig. 4).

The fine structure of the epidermal melanocyte

The fine structure of the melanocytes is extremely varied, probably due to the cyclic activity of the cells. It is possible to see oval and oligodendritical cells either without melanosomes or with only a few premelanosomes. Microfilaments and microtubuli are weakly developed in these cells and, as a rule, are found centrally in the pericaryon, near to the nucleus (Fig. 5). Presumably this form represents dormant melanocytes (Szabo, 1967*a*; Fitzpatrick et al.; Jimbow, Pathak & Fitzpatrick, 1973) (Fig. 2*a*). In other cells, large quantities of microfilaments can be observed radiating at right angles to the nucleus membrane, passing through the pericaryon and continuing out into the dendrites (Fig. 6). At times, near to the nucleus, microtubuli are aligned parallel to the microfilaments (Fig. 7; Fig. 2*b*). In more dendritic cells the microfilaments are aligned peripherally, far from the nucleus and near to the Golgi zone, where large numbers of melanosomes are seen at various stages of development (Fig. 8). The cell continues to develop dendrites and produces more melanosomes, which gradually mature and are transported from the pericaryon out into the dendrites (Fig. 9; Fig. 2*c*). In the dendrites large quantities of microfilaments and microtubuli can be observed aligned parallel with the longitudinal axis of the dendrite, and among these organelles melanosomes are to be found. Some melanosomes are placed in small pouches on the dendrite near the cell membrane (Fig. 10). This presumably, is part of the transference process of melanosomes from melanocyte to epidermocyte. The result of transference of melanosomes can be seen in Fig. 11, where a light and adendritic melanocyte is in contrast with the epidermocytes, which are heavily laden with melanosomes and melanosome complexes.

As far as the morphology of the melanosomes is concerned, we have observed the well-known normal maturing stages (Birbeck & Barnicot, 1959; Drochmans, 1966; Jimbow & Fitzpatrick, 1973; Breathnach, Gross & Martin, 1973; Allegra, 1974; Hunter, Mottaz & Zelikson, 1970). In 2 melanocytes, one from the face and the other from the back, the presence of huge melanosome complexes within the pericaryon was observed (Fig. 12). These complexes, which contained large quantities, of melanosomes of varying sizes were, apparently, without a limiting membrane. On the contrary, they were surrounded by thick bunches of microfila-

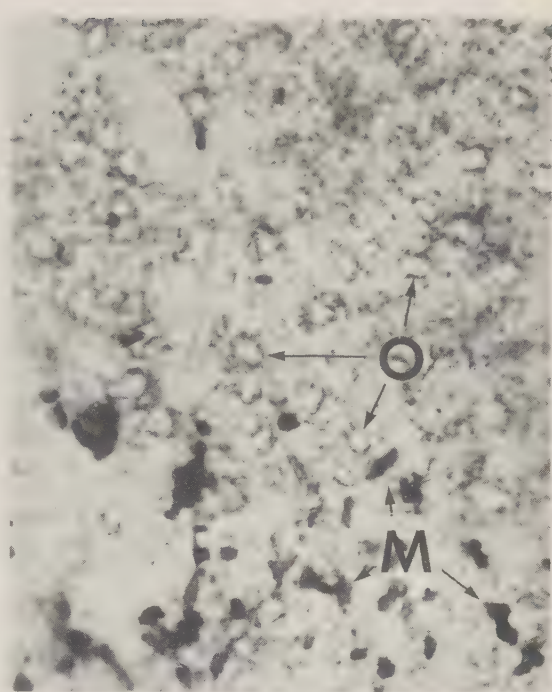


Fig. 1. Epidermal preparation and DOPA treatment. Many of the melanocytes have been torn out from the epidermis under preparation procedures. One can see many holes (O), and remaining melanocytes (M). $\times 500$.

ments. The development and quantity of other cell organelles, mitochondria, ribosomes and endoplasmic reticulum corresponded to the activity level of the cell. No pathological alterations were observed in their morphology.

DISCUSSION

No pathological condition was found in any patient that could influence skin pigmentation. Cochran (1971) has demonstrated that neither basocellular nor squamocellular carcinoma of the skin influences the number of epidermal melanocytes. The DOPA-staining of epidermal sheets has been used in studies of the epidermal melanocyte system in a normal physiological state (Staricco & Pinkus; Mitchell; Szabo, 1967*a*; Fitzpatrick & Breathnach; Billingham, 1948; Becker, Fitzpatrick & Montgomery, 1952; Pinkus, Staricco, Kropp & Fan, 1959; Szabo, 1959; Billingham & Silvers, 1960; Glimcher, Kostick & Szabo, 1973; Srisopark, 1976) as well as in a number of pathological conditions (Kawamura,

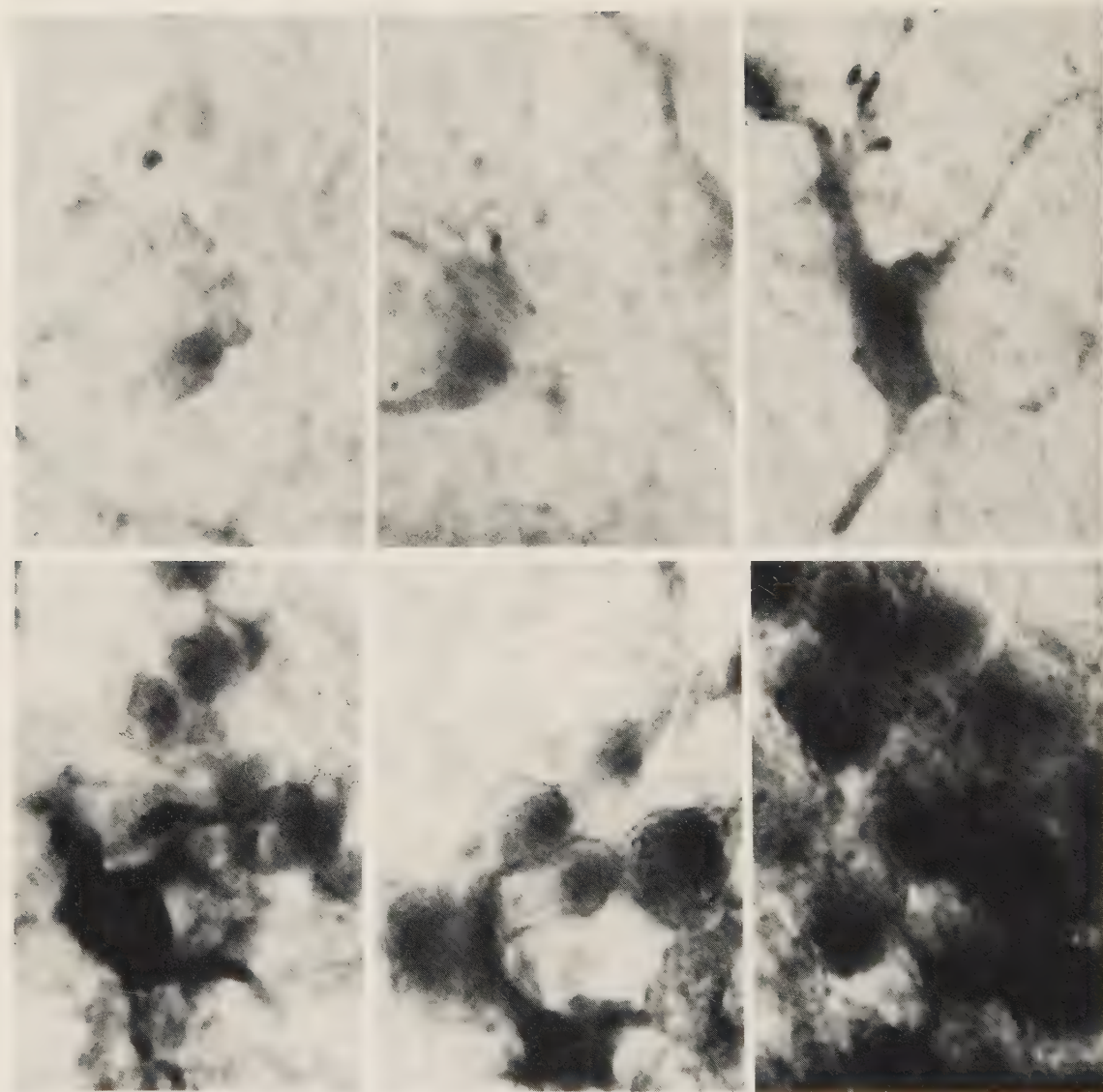


Fig. 2. Epidermal preparation and DOPA treatment. Various morphological forms of melanocytes depicting cell cycle.

Ikeda, Hori, Obata & Nümura, 1971; Fitzpatrick et al.; Drzewiecki; Tsukada, 1974; Papa & Kligman 1965; Takahashi, 1976; Jimbow, Fitzpatrick, Szabo & Hori, 1975; Pinkus et al.). The method is also used for studies of melanocytes under the influence of both external factors (Bleehen, 1976; Frenk & Kocsis, 1973; Miller-Milinska & Staricco, 1963; Staricco, 1957) and internal hormonal factors. (Snell & Lerner, 1966; Snell, 1964). On the basis of

investigations of more than 150 epithelial sheets in an earlier study (Drzewiecki) and 54 epithelial sheets in this present material, it is our opinion that the method generally is effective, but at the same time there are the following weaknesses:

- 1) During the separation procedure a number of melanocytes are torn out of the epithelium (Fig. 1).
- 2) Some cells are deformed by the separation of the epidermis from the dermis.

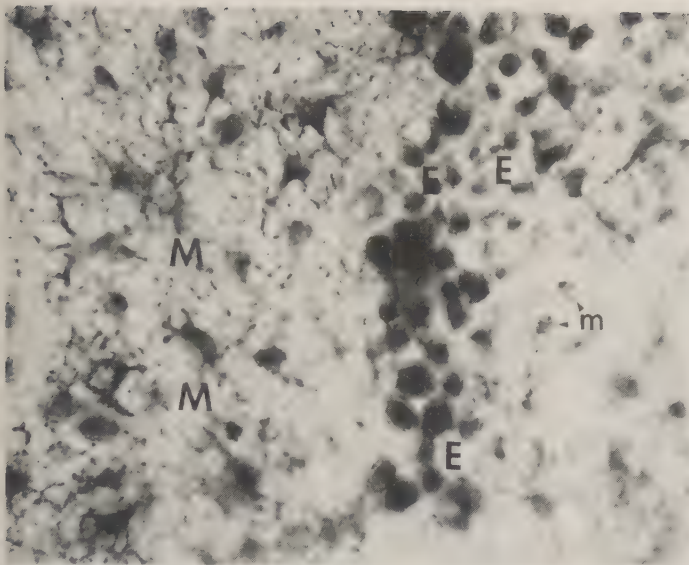


Fig. 3. Epidermal preparation and DOPA treatment. Polygonal, dendritic melanocytes (*M*), adjacent to area with intensely stained epidermocytes (*E*), and fading melanocytes (*m*). $\times 490$.

3) In epidermal sheets with high ridges and deep valleys it is sometimes difficult to count the melanocytes as several of them overlap each other on the steep ridge slopes.

4) The distribution and the density of cells in a given epidermal sheet are occasionally irregular, presumably because of the physiological cycle of the melanocytes.

Those conditions can influence the counting results; and in 5 patients significant differences were found in the number of melanocytes in the three epithelial sheets from the same specimen.

Becker et al. have shown that there are no variations in the melanocyte count in different anatomical regions. However, a number of later studies have demonstrated quite pronounced regional and individual differences (Staricco & Pinkus; Mitchell, 1963; Szabo, 1967*a*; Pinkus et al.; Szabo, 1959; Hu, Staricco, Pinkus & Fosnaugh, 1957). Both regional and individual differences were also present in our material. One of the factors influencing the melanocyte count in the epidermis is the thickness of the epithelium and the development of the dermal surface (number, height and breadth of the ridges). There are nearly always more melanocytes on the ridges than in the valleys. Those melanocytes found in the valleys are, as a rule, large, polygonal and dendritic. Similar observations have been made in other studies (Billingham; Staricco & Pinkus; Szabo, 1959). Staricco & Pinkus are of the opinion that the uneven distribution is accentuated by an optical

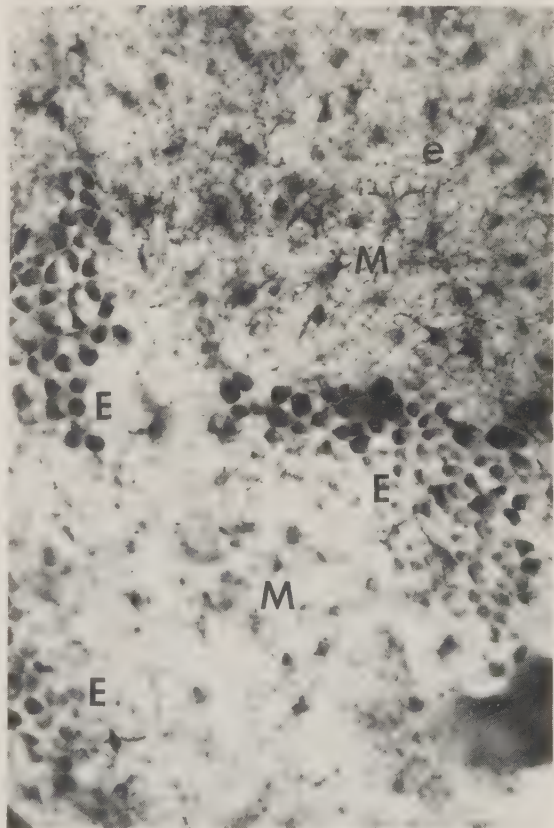


Fig. 4. Epidermal preparation and DOPA treatment. Areas with intensely stained epidermocytes and few melanocytes (*E*), (*M*), alternate with areas with weakly stained epidermocytes (*e*), and many strongly stained dendritic melanocytes (*M*). $\times 150$.

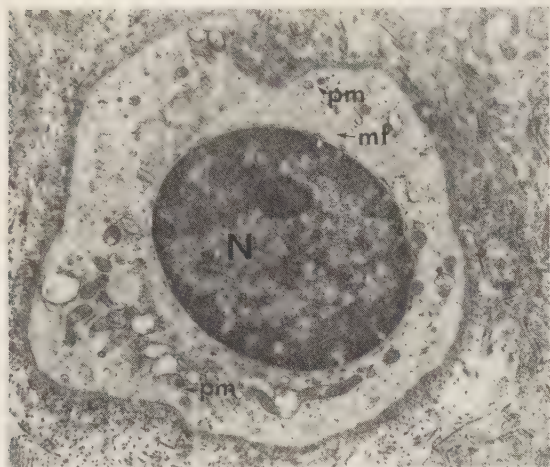


Fig. 5. The epidermal melanocyte. A few premelanosomes (*pm*) and a bundle of microfilaments (*mf*) can be seen near nucleus (*N*). TEM $\times 7300$.

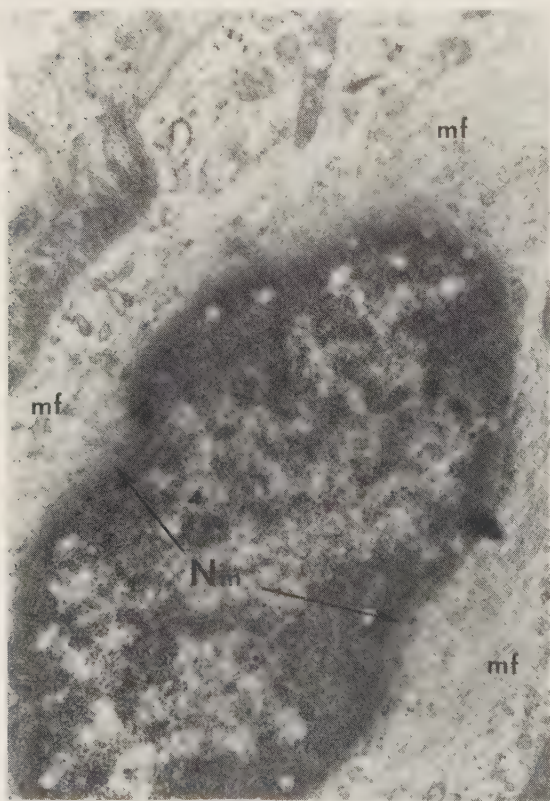


Fig. 6. The epidermal melanocyte. Many microfilaments (*mf*), radiating from the nucleus membrane (*Nm*) can be seen. These microfilaments are then aligned parallel to the nucleus and cell membrane. TEM $\times 12350$.

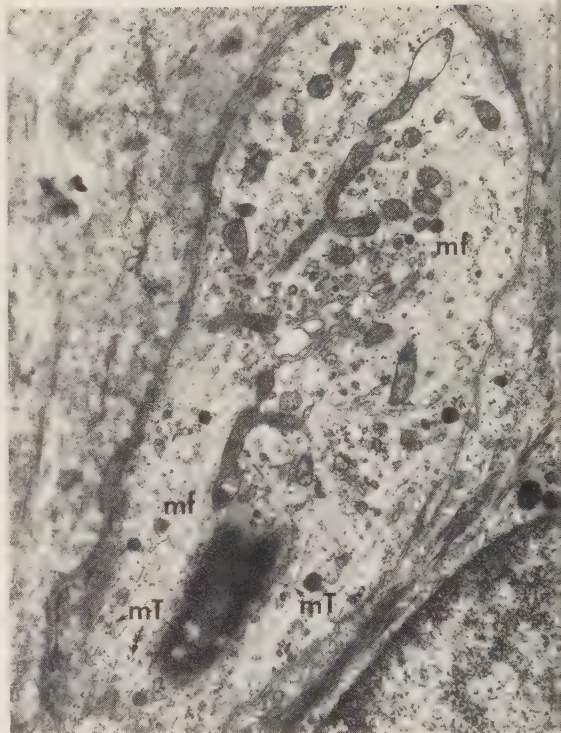


Fig. 7. The epidermal melanocyte. Many microfilaments (*mf*) radiate from the nucleus membrane, and align in bundles in the cytoplasm. Many microtubuli (*mT*) run parallel to the microfilaments. TEM $\times 13400$.

illusion inasmuch as more cells are counted in undulating epithelium than in flat epithelium. However, Szabo (1959) has cast doubt on this theory for he has shown that in flat epithelium from the cheek there are more melanocytes than in highly undulating epithelium from the back. Even though the number of melanocytes in different regions is genetically conditioned (individual differences) and subjected to constant external (Bleehen; Frenk & Kocsis; Miller-Milinska & Staricco) and internal influences (Snell & Lerner; Snell), we are of the opinion that the local anatomical factors also play an important role in the distribution of the melanocytes. Together with a certain number of epidermocytes, the melanocyte forms a functional unit, the epidermal melanin unit (Fitzpatrick & Breathnach).

In a normal physiological condition the relationship between the melanocyte and the number of epidermocytes is quite constant (Szabo, 1959; Szabo 1967a; Fitzpatrick & Breathnach; Fitzpatrick et al.), viz. when the number of epidermocytes in-

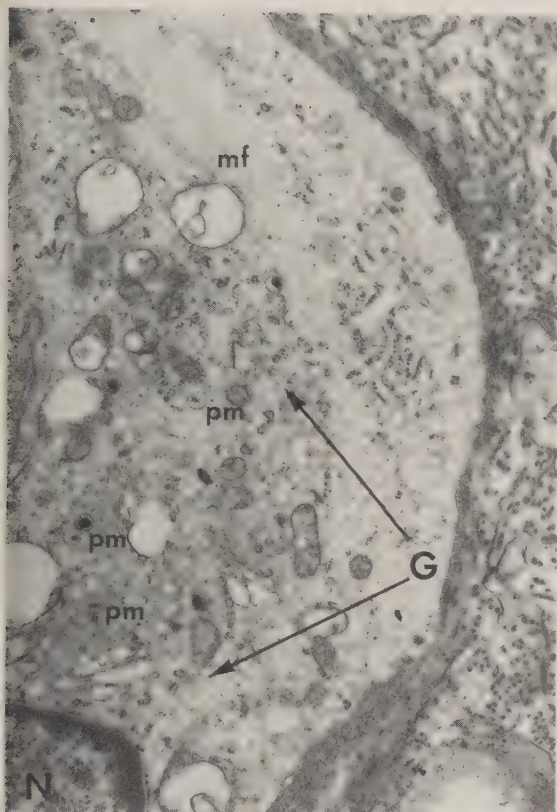


Fig. 8. The epidermal melanocyte. A prominent Golgi area (G) can be seen with many premelanosomes (pm), and a bundle of microfilaments (mf) distant from the nucleus (N). TEM $\times 12\,500$.

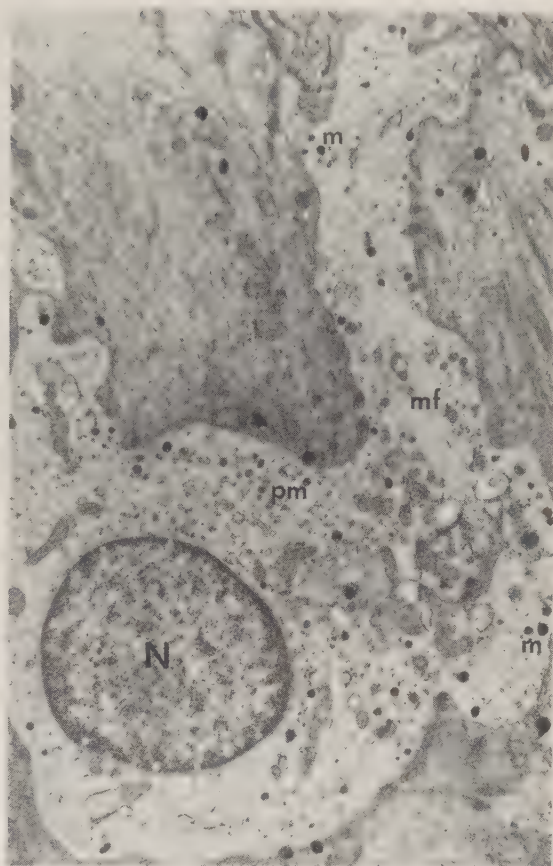


Fig. 9. The epidermal melanocyte with many dendrites. Microfilaments (mf) are seen in the dendrites, but not around nucleus (N). There are many melanosomes (m) and premelanosomes (pm). TEM $\times 7\,300$.

increases the number of melanocytes also increases. This state is reflected by the distribution and morphology of the melanocytes between the ridges and valleys of the epidermis. There are a greater number of epidermocytes in ridges and these are more active (Billingham & Medawar, 1953; Snell & Bischitz, 1963). Thus, there will also be a greater number of melanocytes. A pool of epidermocytes belonging to a certain melanocyte will in the valleys stretch over a larger area. For the same reason the melanocytes are more polygonal and heavily dendritic in form. Staricco & Pinkus, Szabo (1959, 1967a), Mitchell (1963) and Srisopark have claimed that the number of epidermal melanocytes is the same for different races. However, other studies indicate a certain race difference (Mitchell, 1968; Szabo, 1967b). Our results correspond well with those of the well known studies (Staricco & Pinkus; Mitchell, 1963; Szabo, 1967a, Szabo, 1959). In our material

there was a mean of $1\,548 (\pm 50)$ SE cells per 1 mm^2 of epidermal surface: this figure agrees with the mean stated by Szabo (1967a) ($1\,560 \pm 110$ cells/ mm^2).

Both DOPA and TEM studies produce an extremely varying morphological picture of the melanocyte. Close examination of numerous morphological variants suggests that the melanocyte is an active and dynamic cell, probably subjected to a biological cycle. This phenomenon has also been observed by other authors (Staricco & Pinkus; Mitchell, 1963; Szabo, 1967a; Glimcher et al.). Szabo (1959) states that the "tyrosine reaction also shows variation from cell to cell". The growth of the cell probably begins with the formation and establishment of the cytoskeleton (microtubuli and microfilament complexes) (Figs. 6 and 7). A well-

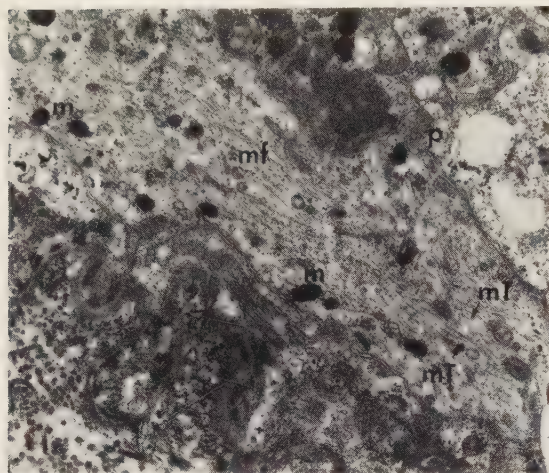


Fig. 10. Part of the dendrite, with parallelly aligned microfilaments (*mf*) and microtubuli (*mt*) and melanosomes (*m*). One of the melanosomes is placed within a pouch of the dendrite (*p*). TEM $\times 12800$.

functioning cytoskeleton is essential for upholding the dendrital form of the cell and the intracellular transport of melanosomes (Magun, 1973; Wikswo & Szabo, 1972; Wikswo, 1973; McGuire, Moellmann & McKenon, 1972; Jimbow et al.; Moellmann, McGuire & Lerner, 1973; Jimbow & Fitzpatrick, 1975; McGuire & Moellmann, 1972; Sauk, White & Witkop, 1975; Kitano, 1976) (Fig.

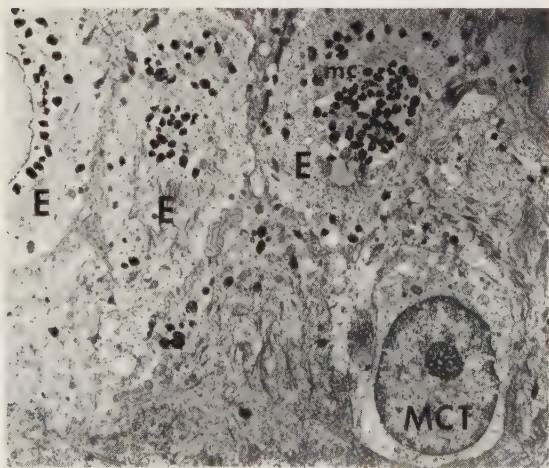


Fig. 11. The epidermis with malpighian cells (*E*) laden with melanosomes and melanosome complexes (*mc*) and oval melanocyte (*MCT*) without melanosomes in cytoplasm. TEM $\times 3600$.

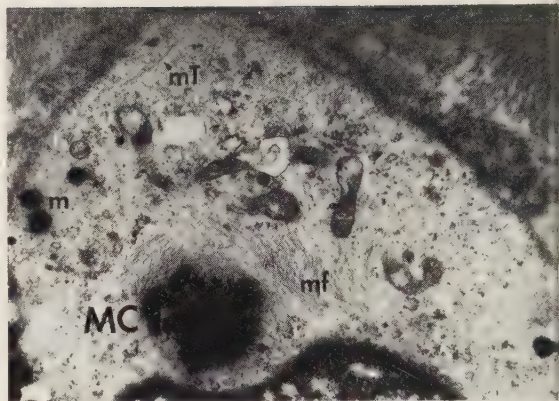


Fig. 12. The melanocyte with a huge melanosome complex (*MC*), which is surrounded by broad bunch of microfilaments (*mf*). Note absence of limiting membrane. Melanosomes (*m*), microtubuli (*mt*). TEM $\times 12350$.

2c). Simultaneous with the construction and upholding of the cytoskeleton, the melanogenesis increases resulting in the production of melanosomes (Fig. 8). With the help of the cytoskeleton (microfilaments) these are transported out into the dendrites (Fig. 10) (Jimbow & Fitzpatrick, 1975; Jimbow et al.; Wikswo & Szabo; Magun) whence they are transferred to the epidermocytes (Fig. 11). The transference mechanism is not universally accepted. One theory claims that the melanocyte actively sprays melanosomes into the epidermocytes (Masson, 1948; Drochmans, 1961; Mishima, 1966). Another theory proposes that the melanosomes are released to the intercellular spaces where they are phagocytosed by epidermocytes (Swift, 1964; Wolff, Jimbow & Fitzpatrick, 1974; Kukita, Sato & Ito, 1974; Wolff, 1973). Finally, a third cytophagocytic theory suggests that the epidermocyte phagocytes part of the melanocyte's dendrite, which is filled with melanosomes (Cruikshank & Harcourt, 1964; Mottaz & Zelickson, 1967; Okazaki, Uzuka, Morikawa, Toda & Seiji, 1976). Our investigation does not enable us to say whether the cell cycle described above begins or ends with mitosis or how quickly this takes place. Melanocytes are capable of dividing themselves, and they preserve their "cytologic characteristics" during division (Sato, Nishijima, Hiraga & Hidano, 1975; Rosdahl & Szabo, 1976; Jimbow, Roth, Fitzpatrick & Szabo, 1975; Giacometti, Montagna, Bell & Hu, 1972). However, we have not observed melanocytes in mitosis in our normal material.

We have found that several neighbouring melanocytes become active in an area of the epidermis (Fig. 4). Pinkus et al. are of the opinion that "the melanocytes are not influenced by mitotic activity of epidermocytes". On the other hand, Hadley & Quevedo (1966) are of the opinion that "ultraviolet light stimulates melanogenesis indirectly by inducing proliferation of keratinocytes". We have the impression that the cyclic function of the melanocyte is presumably dependent on the mitotic activity of the epidermocytes since the melanocyte has to supply a new population of epidermocytes with melanosomes.

In our normal material we found 2 melanocytes with huge melanosome complexes. These ultrastructural alterations have been observed in pathological conditions of the skin (Takahashi; Zelickson, Windhorst, White & Good, 1967; Kawamura, Ikeda, Hori & Obata, 1966) and after ultraviolet influence (Zelickson, Mottaz & Hunter, 1972). Huge melanosome complexes can, however, also occur in melanocytes in normal skin.

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THE SURFACE MORPHOLOGY OF THE MELANOMA CELL

A Scanning Electron Microscope Study on the Primary Cutaneous Melanoma

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Abstract. The surface morphology of the melanoma cell in situ is summarized with regard to a study undertaken to elucidate the appearance of melanocytes and their differentiation in the surroundings of the malignant melanoma. Despite a non uniform cell pattern, several characteristic changes were found. The cells were 2-3 times the size of normal melanocytes, a number being differentiated by dendrites. The surface of the cell was marked by numerous extensions, a number of which had differentiations that indicate a special function, though some smooth-surfaced cells were also found. It is possible that cell surfaces with many bulges and extensions are an expression of an unstable, undulating surface which could be of significance for the uncontrolled growth and metastasis of tumours.

The preferred treatment for the cutaneous malignant melanoma is broad excision of the skin and subcutaneous tissue at some distance from the macroscopic margin of the tumour. The principle is based on a number of clinical observations which show that the excision of the tumour with a narrow margin often leads to subsequent spreading in the form of local cutaneous metastases.

Theories presented by Petersen, Bodenham & Lloyd (1962) and Olsen (1966) consider the malignant melanoma as being capable of activating the epidermal melanocytes and that their transformation could be the cause of local metastases, whose presence would worsen the patient's prognosis considerably.

In the light of the above, a morphological analysis

of biopsy material from both cutaneous malignant melanoma and its surroundings has been commenced in order to acquire a better understanding of the differentiation of melanoma cells, and melanocytes in the vicinity of the tumour.

In an earlier study, Drzewiecki & Kj rgaard (1977) summarized the surface morphology of the normal melanocyte. The present paper summarizes surface morphology of malignant melanoma cells.

According to Clark, From, Bernadino & Mihm (1969) and McGovern & Lane Brown (1969) the cutaneous melanomas can be divided into three groups based on clinical and histological growth patterns correlated with their biological behaviour. The types are designated by Clark et al. as: "Lentigo maligna melanoma", "superficially spreading melanoma" and "nodular melanoma". Electron microscopic studies show ultra-structural differences not only between cells in these groups but also between cells within the same tumour (Hirone, Nagai, Matsubara & Fukushima, 1971; Clark, Heggeler & Bretton, 1972). Despite these morphological variations some patterns appear that permit the allocation of cells into one of four types. Type I is characterized by a relatively minor disorganisation of the ultrastructure of the cell; in types II and III the inner structures are increasingly disorganized; and in type IV the cell appears completely disorganized and has lost all resemblance to the melanocyte (Clark et al., 1972).

The above studies concentrated exclusively on the inner structure of the cell. Very little information is available concerning the surface morphology

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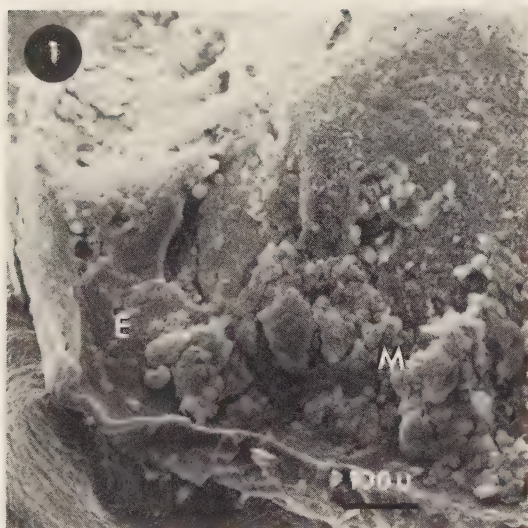


Fig. 1. SEM, $\times 100$. Biopsy from malignant melanoma. The epithelium is seen in the lower part of the field (E). The malignant cells (M) invade the epithelium.

of the melanoma cell. Comparative electron microscope studies on Hutchinson's melanotic freckle and premalignant melanosis reveal an increasing number of bulges on the cell surface (McGovern & Lane Brown). Considering this electron microscopic difference, it is tempting to study the surface morphology of the cells, since inter-cell contact and influence must affect this.

The importance of the cell surface is apparent from *in vitro* studies on cell cultures transformed from normal to malignant cells. After transforma-

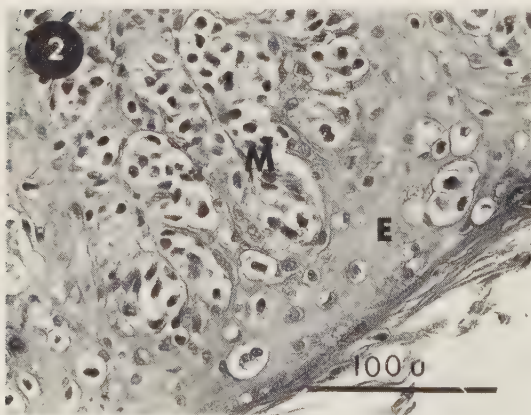


Fig. 2. Light mic., $\times 250$. Section from the same tumour as in Fig. 1. Tumour cells (M) in the shape of single cells and cell nests invade the epithelium (E).

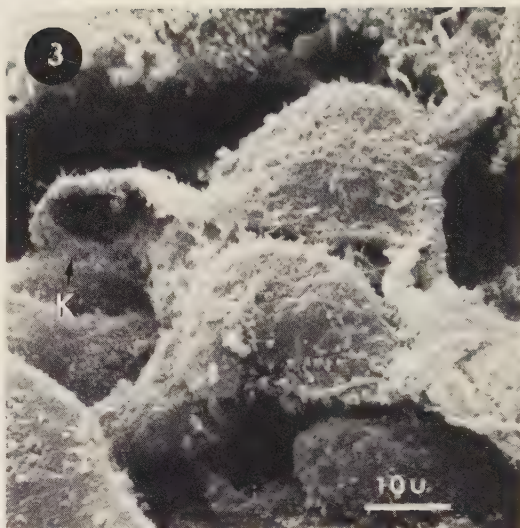


Fig. 3. SEM, $\times 1000$. Dendritic tumour cells. One of the dendrites contacts a neighbour cell (K).

tion the surface has changed (Porter, Todaro & Fonte, 1973; Yamaguchi & Weinstein, 1975; Hale, Winkelhake & Weber, 1975). Additionally, it is generally recognized (Lewis, 1972; Hellström, Hellström, Sjögren & Warner, 1973; Kåresen, 1974) that the malignant melanoma is an immunologically active tumour. The cell surface is of importance for the antigenic properties of the cell (Stoker, Thornton, Riddle, Birg & Meyer, 1972; Hellström, War-

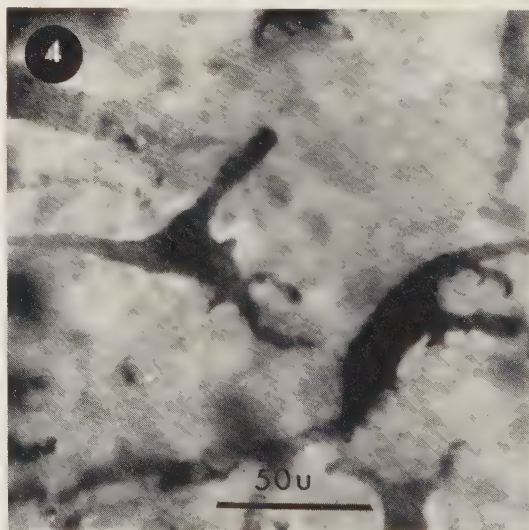


Fig. 4. Light mic., $\times 400$. DOPA staining. Epithelial sheet from the same tumour as in Fig. 3, with dendritic tumour cells.

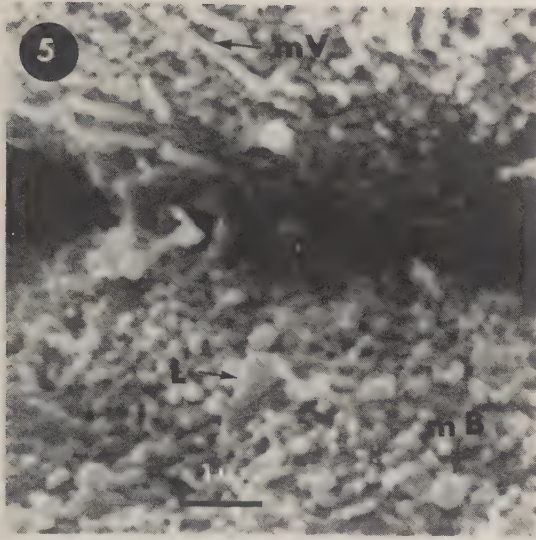


Fig. 5. SEM, $\times 10\,000$. Two serial melanoma cells. The surfaces are uneven, covered with microblebs (*mB*), microvilli (*mV*) and some lamellae (*L*).

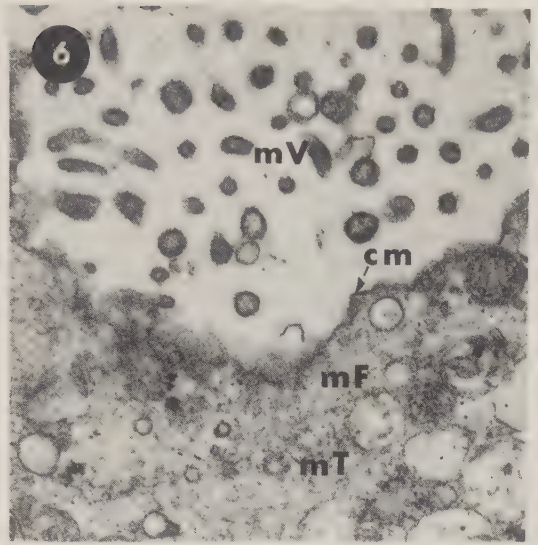


Fig. 6. TEM, $\times 18\,000$. Section through melanoma cell from the same biopsy as in Fig. 5. The cell membrane (*cm*) is irregular. Microvilli (*mV*) can be seen above the membrane. Below the membrane, numerous intermingled microtubules (*mT*) and microfilaments (*mF*) can be seen.

ner, Hellström & Sjögren, 1973; Wartiovaara, Linder, Ruoslahti & Vaheri, 1974).

Scanning electron microscopy (SEM) has been used in the present study on the morphology of the cell surface—a method of investigation not previously employed in the study of the melanoma cell in biopsy material.

METHOD

Six patients with primary malignant melanoma were examined. In all cases the tumour developed in a birthmark over periods ranging from 9 months to 2 years. The tumour biopsy was taken immediately after the tumour had been surgically excised, with a minimum of 5 cm distance. Each of the specimens was divided into 5 parts: for scanning electron microscopy (SEM), transmission electron microscopy (TEM), haematoxylin-eosin staining (HE), DOPA staining, and the fifth part was incubated in a solution of EDTA+T199 for the purpose of separating the epidermis from the dermis. For details of these methods, see Drzewiecki & Kjærgaard.

RESULTS

Based on the HE staining, four of the tumours were classified as "superficially spreading malignant melanomas", and two as "nodular melanomas". The superficially spreading melanomas invaded the dermis in varying degrees but maximally to the reticular layer (level 3). Similarly, melanoma cells were found to have invaded the epidermis in the

form of single cells as well as nests of cells (Fig. 2). Only a few abnormal mitoses could be observed. The two nodular melanomas invaded all layers of the dermis (level 4). One of them was peduncular and exophytic. The malignant cells in this tumour were fusiformed, and only a few abnormal mitoses were observed. The melanin deposits in the tumour region were large. No tumour invasion of the epidermis was found.

Cell structure as evaluated by TEM and SEM studies

The superficially spreading malignant melanoma. The ultrastructure of the cells varied greatly, particularly as far as the melanosomes were concerned, where the development pattern and morphology were marked by disorganization. On the basis of the ultrastructural appearance of the cells it would seem possible to use Clark's classification. Types II and III were dominant.

The plasma membrane varied according to the siting of the cells. Those melanoma cells placed between epidermocytes had most commonly a smooth surface. Cells found lying freely in the stroma of the tumour or in the ulcerated surface usually had a heavily folded and irregular surface, with formation of blebs, microblebs and microvilli.

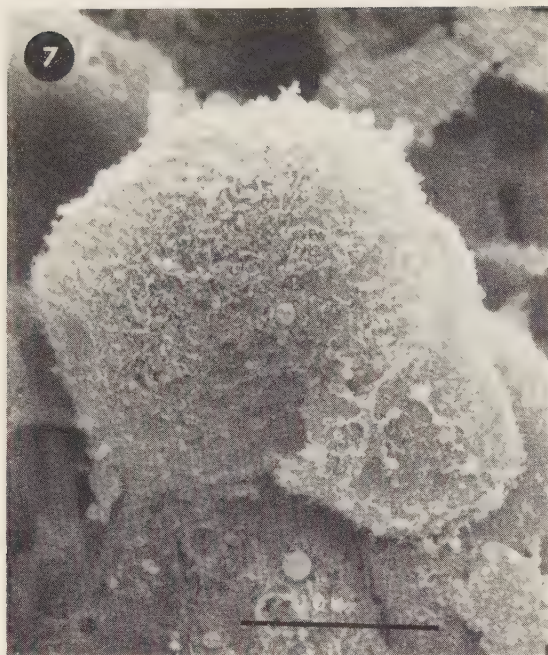


Fig. 7. SEM, $\times 3000$. Adendritic and rounded-off malignant melanoma cells with numerous microvilli (mV) and microblebs (mB).

In sites where many microvilli were found, accumulations of intermingled microtubules and microfilaments were seen beneath the cell membrane (Fig. 6). In the microvilli, 1–2 microtubules (one as a rule) could be observed (Fig. 8). The blebs contained an amorphous substance or incompletely developed melanosomes (Fig. 9).

By SEM examinations of the same biopsies the malignant melanoma cells could be identified (Fig. 1). The size of the cells varied from 10 to 30 μm —they were thus 2 to 3 times the size of normal melanocytes. The cells were spherical or oval in shape (Fig. 7), and some of them had developed dendrites that were usually short with few branches (Figs. 3, 4). The dendrites, the length of which varied, could be observed in their course in the narrow spaces between the melanoma cells. Other dendrites contacted surrounding melanoma cells without forming specialized contact organelles. The cell surface was in most instances highly irregular with numerous bulges and projections in the shape of microvilli, microblebs, conglomerates of microblebs as well as blebs (Fig. 5). The spherical cells usually had more surface bulges than did the dendritic ones. The microvilli have a diameter of

0.1–0.3 μm , most often 0.1 μm , and their length is varied. They project from the cell surface, twist upon it or stand out erectly and, in a number of instances, form contact with neighbouring melanoma cells (Figs. 5, 8). In the investigated preparations there were some smooth melanoma cells, though these were few in numbers.

The nodular malignant melanoma. TEM examination revealed that one of the two tumours was formed of greatly varying cells, dominated, nevertheless, by the low-differentiated Clark cell-type IV. The second tumour, which clinically was exophytically proliferating, proved to be constructed of an impressive number of high-differentiated cells. These were mostly cells of type I. Most of them were dendritic and contained many completely melanised melanosomes. Immediately beneath the plasma membrane, microtubules and microfilaments with parallel courses were observed (Fig. 10).

The first of the two above nodular melanomas was found by SEM examination to be constructed of large rounded cells, some of them displaying somewhat differentiated dendrites. The morphology of the cell surface varied as regards the number

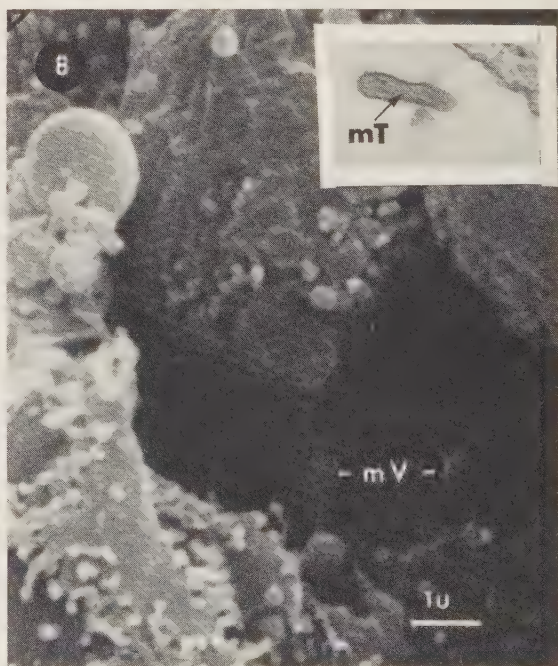


Fig. 8. SEM, $\times 10000$. Malignant melanoma cells. Microvilli (mV) contact neighbouring cells. (Inset) TEM section through microvilli containing microtubules (mT).

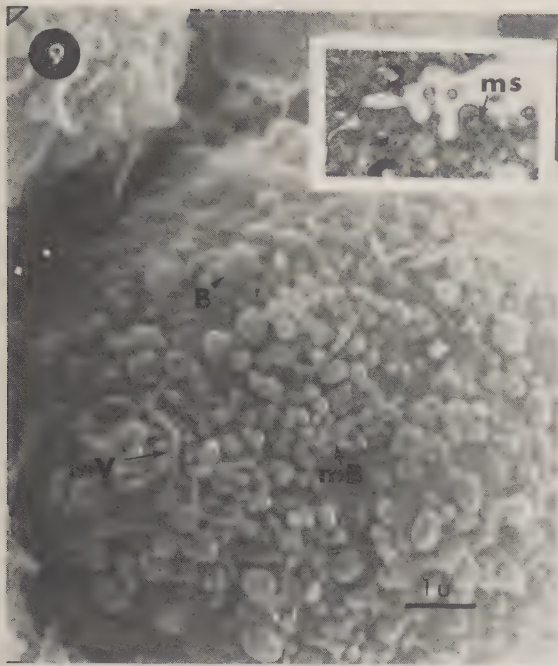


Fig. 9. SEM, $\times 10\,000$. Malignant melanoma cell. The surface is covered with microblebs (mB), microvilli (mV) and blebs (B). (Inset) TEM section through the cell surface with blebs. Blebs contain either an amorphous substance or some melanosomes (ms).

of surface structures. The presence of cells with irregular surfaces was far more frequent than was the case with the superficial melanoma. The second nodular melanoma (clinically exophytically proliferating) was constructed of oblong bipolar cells with thick, stumpy dendrites (Fig. 10). Some of the dendrites were extremely long and slender, and twisted in among the neighbouring cells. In this type of tumour it was possible to observe dendrite/cell contacts between the melanoma cells, but no specialized contact organelle could be demonstrated. In general the cell surface was smooth.

DISCUSSION

Despite the highly varied morphological picture of the malignant cells in the melanomas investigated by TEM, it was, nevertheless, possible to observe in the individual tumours a certain morphological tendency that permitted the classification of the cells according to the four Clark types. This study thus seems to support the theory of a relationship between the intracellular ultrastructure of the tumour cell and the biological behavioural pattern

of that tumour, (Clark et al.; McGovern et al.; Hirone et al.). Thus the superficially spreading malignant melanoma was found to be predominantly constructed of relatively highly differentiated cells of types II and III, while one of the nodular malignant melanomas was formed of low differentiated cells of type IV. It should be added that this tumour had already metastasized to the regional lymph nodes. However, the cells found in the exophytically proliferating malignant melanoma were unexpectedly highly differentiated and of Clark type I.

In recent years cell research has shown a tendency to focus on the ultrastructure of the cell membrane in malignant cells, with attention to the significance of this for the explosive, uncontrolled growth as well as antigenic properties against the host organism (Patterson, 1974; Dermer, Lue & Neustein, 1974; Berlin, Oliver, Ukena & Yin, 1975; Nicolson, 1975). SEM studies of viral transformed fibroblasts *in vitro* show that the cells piled up, and that concomitantly the surface became irregular as a consequence of the projections (blebs and microvilli) (Hale et al.; Porter et al.). Scanning

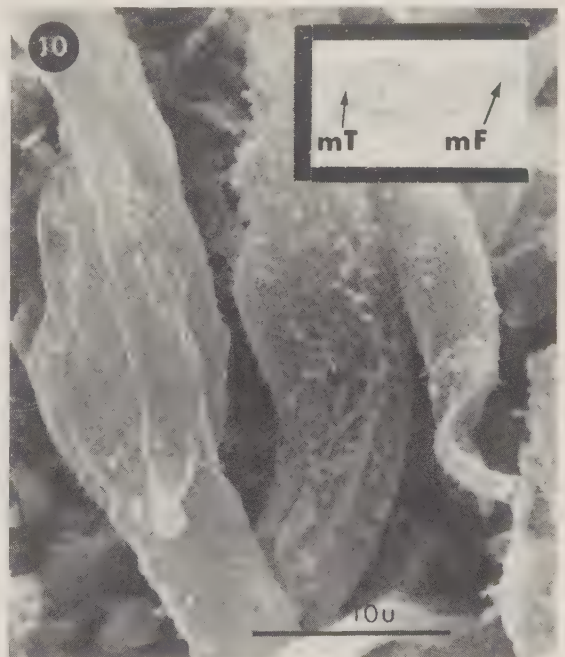
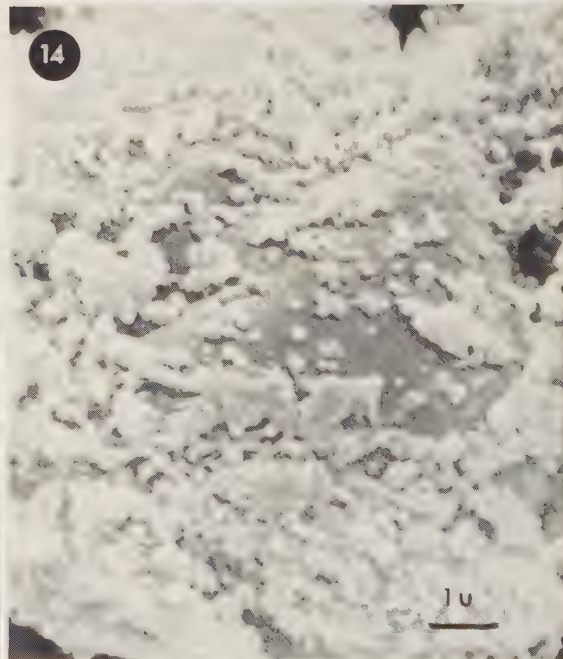


Fig. 10. SEM, $\times 3\,000$. Malignant melanoma cells are oblong and dendritical. The surface is relatively smooth. (Inset) TEM. The cell surface is smooth. Below the cell membrane, microtubules (mT) and microfilaments (mF) are organized in rows parallel with the cell membrane.



Figs. 11-14. (11) SEM, $\times 8800$. Malignant melanoma cell with smooth surface. (12) SEM, $\times 8800$. Malignant melanoma cell. Surface with blebs. (13) SEM, $\times 8800$. Malignant melanoma cell. Surface dominated by

microblebs and microvilli. (14) SEM, $\times 8800$. Malignant melanoma cell. Surface with conglomerates of microblebs.

microscopy studies on the human melanoma cell *in situ* have not previously been undertaken.

The present study revealed that the majority of malignant cells had considerably more surface extensions than normal melanocytes (Drzewiecki & Kjægaard).

The surface morphology of the investigated cells varied highly but also here it has been possible to observe a certain pattern. It was possible to isolate four types of cell surface: Type I, cells with a smooth surface (Fig. 11); Type II, cells where the surface is marked by bleb-like bulges (Fig. 12); Type III, cells where the surface consists of agglomerates of microblebs (Fig. 14); and Type IV, cells where the surface is dominated by projections in the form of microblebs, microvilli and lamellae (Fig. 13).

However, it should be underlined that cells displaying all of the above structures on the same cell surface can be found (Fig. 9).

It is not possible at this point by comparing TEM and SEM studies to state whether the enumerated types of cells bear any relationship to the four cell types tabulated by Clark et al. It is possible that these cell surface types depict the four phases in the cell cycle (G^1 , S, G_2 , M). Studies on cell cultures indicate that the surface morphology of the cell is dependent on the cell cycle (Porter, Prescott & Frye, 1973; Sauk, White & Witkop, 1975), so that the mitotic cell is distinguished by many surface projections.

The significance and cause of the numerous surface projections on the malignant melanoma cell still remain unclear. Recent investigations of cell cultures indicate that cyclic AMP (cAMP) could be involved in these alterations. It is considered that cAMP is an essential factor for the formation of microtubules from protein monomers (Hsie & Puck, 1971). Similarly, cAMP is regarded as decisive for the positioning of the microtubules in the cell. Cells where the microtubules are sited beneath the cell membrane and orientated parallel with it have a stabile surface (Porter et al.; Vasiliev, Gelfand, Domnina, Ivanova, Komm & Olshevskaja, 1970). Transformed cells have a low content of cAMP (Otten, Johnson & Pastan, 1971). There is some evidence that malignant melanoma cells also have a low cAMP content (Pastan, Johnson & Anderson, 1975).

If an analog of cAMP is introduced to cultures of transformed cells, their morphology is changed in

the direction of normalization (Johnson, Friedman & Pastan, 1971; Sheppard, 1971). Concomitantly the motility and growth of the cell are reduced (Otten et al. 1972; Johnson, Morgan & Pastan, 1972). If the cAMP content in malignant cells is increased, then the malignancy of these cells decreases in comparison with untreated cells (Kreider, Wade, Rosenthal & Densley, 1975). By transmission microscopy on cells with irregular surfaces in the form of microvilli and blebs it was possible in the present study to observe that the microtubules and microfilaments were sited intertwined without any regular order. Thus, it is possible that an increased number of these surface structures is an expression of an unstable, undulating surface that could be of importance for metastasis, antigenic properties and uncontrolled growth of the tumour.

The cells that were found in this present investigation in the exophytically proliferating malignant melanoma were unexpectedly highly differentiated, of Clark type I. Transmission microscopy revealed that immediately beneath the cell membrane there was an accumulation of microtubules and microfilaments arranged in rows that parallel the cell surface. Investigation of these cells by SEM showed the surface to be smooth and stable.

Observations on individual sections of the investigated malignant melanomas revealed that microvilli contacted the surface of the neighbouring melanoma cells. Cells with microvilli, and with mutual contact via these microvilli, were particularly seen in places where the cells were lying freely, such as in ulcerations. Transmission microscopy revealed that the microvilli contained microtubules. It is therefore possible that microvilli constitute motile elements that ensure mutual cell contact.

Electrophysiological investigations of transformed cells *in vitro* have shown these to be commonly electrically coupled by way of a low resistant junction. Ions and particles with a molecular weight of up to 500 can be exchanged through coupled cells (Johnson & Sheridan, 1971).

Furthermore, it must be assumed that cells with many surface extensions, and thereby a large surface, are particularly active and have an enhanced uptake of metabolites (Folett & Goldman, 1970).

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CUTANEOUS MALIGNANT MELANOMA¹

Studies on the Fine Structure of Cells and Their Surface Morphology in Relation to Tumour Type and Clinical Course

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Abstract. Cutaneous malignant melanoma occurs in three forms: lentigo maligna melanoma, superficially spreading melanoma, and nodular melanoma. The histology, the clinical development and the biological malignancy of these tumours differ. The purpose of the study reported here was to provide a clearer picture of the surface morphology of the malignant cells and to relate their fine structure to both the tumour type and the clinical development of the malignant melanoma. This investigation of 28 tumours from 22 patients of Scandinavian origin shows that at the electron microscope level there is no difference between malignant cells in the invasion nodulus of superficially spreading melanoma and nodular melanoma. The primary tumours were often built up of 2 or 3 differently differentiated cell clones. Metastases were built up of the same cells or cell clones that were found in the primary tumour. In several cases the surface of the malignant cells was folded, and covered with microvilli, microblebs and blebs. These surface alterations could be related in several cases to changes in the cytoskeleton of the cell (microtubuli and microfilament complexes). The clinical course of the malignant melanoma could best be correlated to the histogenetic type of tumour, depth of invasion, nucleus polymorphy and the quantity and arrangement of the microfilament complexes.

The cutaneous malignant melanoma develops in the extensive epidermal melanocyte system. The function and fine structure of the malignant cells clearly differs from the function and fine structure of normal melanocytes (Drochmans, 1966; Hunter, Mottaz & Zelickson, 1970; Hirone, Nagai, Matsubara & Fukushima, 1971; Chang, Saito, Romsdahl & Russell, 1972; Jimbow & Fitzpatrick, 1973; Kukita, Sato & Ito, 1974; Curran & McCann, 1976). However, there are certain similarities for both cell lines, i.e. the development of dendrites and the formation of melanosomes. Alterations in the fine structure of the cell following malignant transformation are often accompanied by alterations in

the surface morphology of the cells (Drzewiecki, 1977). The studies undertaken by Clark, From, Bernardino & Mihm (1969); Clark, Ainsworth, Bernardino, Yang, Mihm & Reed (1975); as well as McGovern (1976) form the basis for the division of malignant melanomas into lentigo maligna melanoma (LMM), superficially spreading melanoma (SSM) and nodular melanoma (NM). The histology, the clinical development and the biological malignancy of these tumours varies.

In recent years studies on the fine structure of the melanoma seem to show that at the electron microscope level too, a difference is to be seen between these tumour types (Cesarini, 1971; Clark, Heggeler & Bretton, 1972; Mishima & Matsunaka, 1975; Bleehe, Hartley & Senior, 1976). On the other hand, there are observations that do not confirm the existence of such a distinction between tumour types (Hunter, Zaynoun, Patterson, McKie, Cochran & Bleehe, 1976). The purpose of the present study was to present a clearer picture of the surface morphology of the malignant cell as well as to relate the fine structure of the malignant cell to the type of tumour and the clinical course of malignant melanoma.

MATERIAL AND METHODS

The study included 28 tumours from 22 patients of Scandinavian origin. The classification of malignant melanomas, their clinical course as well as the distribution of the tumours according to anatomical region are set out in Table I. On the basis of the histological routine investigation, 15 of the tumours were classified as SSM and 8 as NM.

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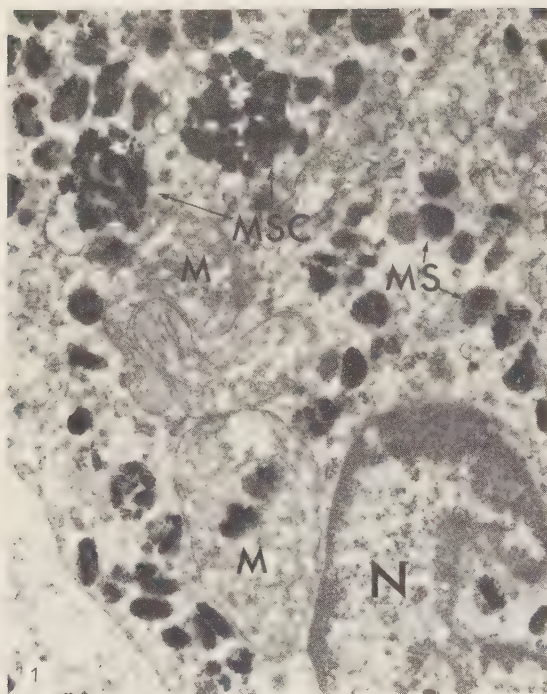


Fig. 1. Part of a malignant melanoma cell with hyperthrophied bizarre mitochondria (M), melanosomes (MS), and melanosome complexes (MSC). Nucleus (N). TEM, $\times 14270$.

One patient, no. XXXI, had developed two primary melanomas with an interval of one year. One of these two was localized to the right leg; this tumour had already metastasized to the skin in the same anatomical region on admission to hospital. The other tumour was localized on the left leg. The first tumour was classified as NM and the second as SSM. Six patients developed metastases to the regional lymph nodes. Two of these metastases were investigated. In 4 patients the tumour metastasized to the skin; three of these metastases were investigated. Five patients died from malignant melanoma between 2 months and 2 years after operation.

All primary tumours were excised at a distance of at least 5 cm. Immediately after excision a biopsy was taken from the invasive nodule of the tumour for transmission electron microscopy (TEM) and scanning electron microscopy (SEM). The preparation procedure for both microscopy methods has previously been described (Drzewiecki & Kjærgaard, 1977). TEM was carried out on all biopsies, while SEM was used on biopsies from 15 patients nos. XX to U-3 (Table I).

RESULTS

TEM evaluation of the cell's inner ultra-structure

Most of the investigated cells had an increased nucleus/cytoplasm ratio. The contour of the nucleus was greatly folded and irregular. Nucleoli, at times multiple, were prominent. No cytoplasmic

Table I. Malignant melanomas. Distribution according to anatomical region, histological classification and clinical course

Patient no.	Anatomical region	Type of tumour	Depth of invasion	Metastasis		
				Dermis	Lymph.node	Dead
X	crus	SSM	II			
XI	crus	SSM	III			
XIII a	dorsum	SSM	III			
XIV	dorsum	NM	IV (pedunculate)		+	+
XV	crus	SSM	IV		+	
XVII	crus	SSM	III			
XVIII	crus	SSM	III			
XX	crus	SSM	III			
XXI	dorsum	NM	(pedunculate)			
XXII	dorsum	SSM	II			
XXIV	dorsum	SSM	III			
XXV	abdomen	SSM	III			
XXVI	dorsum	NM	IV (pedunculate)		+	
XXVII	crus	SSM	IV			
XXVIII	dorsum	SSM	III			
XXIX	crus	SSM	III			
XXX	dorsum	NM	IV	+	+	+
XXXI	crus (R)	NM	V	+		
XXXI b	crus (L)	SSM	III			
XXXIII	crus	SSM	III			
XXXIV	facies	NM	IV	+		+
U-1	dorsum	NM	(pedunculate)	+	+	+
U-3	dorsum	NM	(pedunculate)		+	+

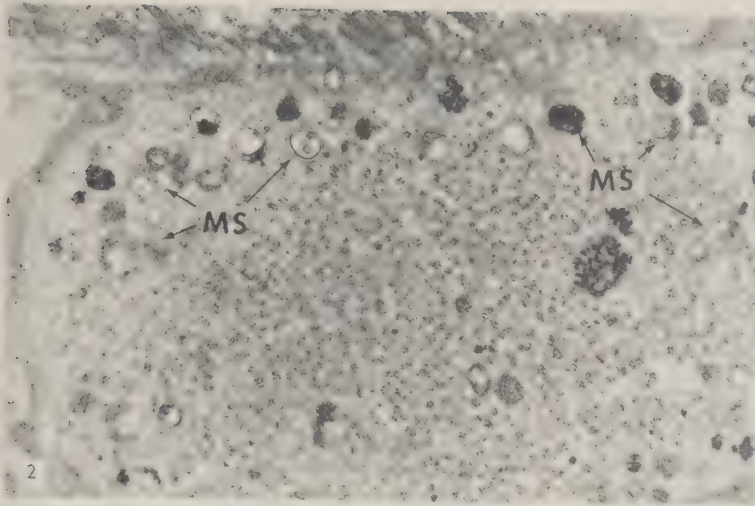


Fig. 2. Part of a malignant melanoma cell. Cytoplasm is filled with ribosomes. Many abortive melanosomes (MS) can be seen. TEM, $\times 13\,630$.

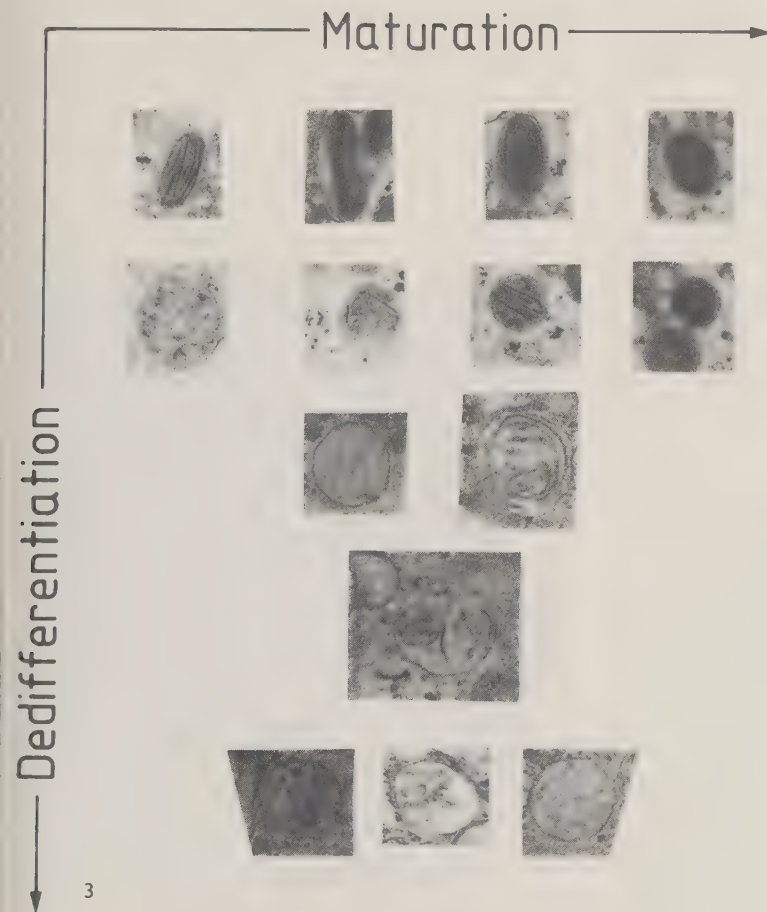


Fig. 3. Various morphological structures of the melanosomes of malignant melanoma cells.

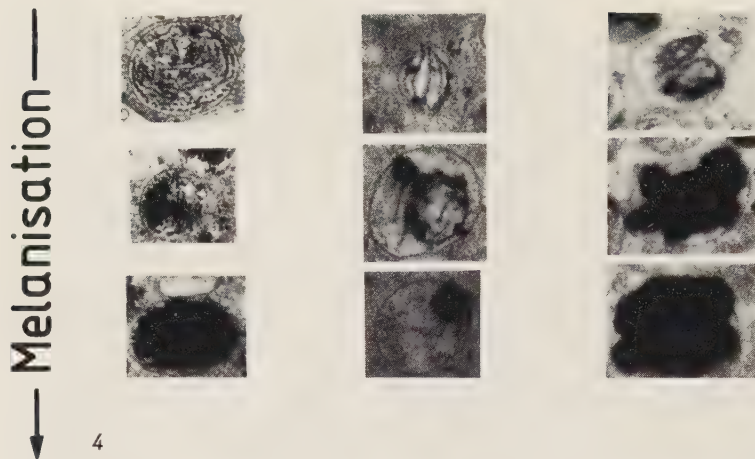


Fig. 4. Various ways of melanization of the melanosomes of malignant melanoma cells.

inclusions were found in any of the nuclei. In 7 of the primary tumours, cells with multilobular nuclei were found (Figs. 6, 8). Of these 7 primary tumours, 5 were NM and 2 were SSM. All the above nodular melanomas metastasized either to the regional lymph nodes or to the skin (patients XXVI, XXX, XXXI, XXXIV and U-1 (Table I)). Neither of the 2 SSM metastasized. The investigated malignant cells had an increased number of hypertrophic mitochondria. In 8 tumours bizarre forms of mitochondria were discovered (Fig. 1). Of these tumours, 3 were classified as NM and 5 as SSM. Most of the investigated malignant melanoma cells had a prominent Golgi area as well as prominent endoplasmic reticulum. Moreover, in many cells an increased number of free ribosomes and polyribosomes were found which filled a great part of the cytoplasm (Fig. 2).

All malignant melanoma cells produced melanosomes, which either had a structure typical of normal melanosomes or a structure more or less similar to normal melanosomes. In the apigmented tumours mainly pre-melanosome forms were found, with weak signs of melanization. From a morphological point of view melanosomes can be divided into fibrillar, granular and vesicular forms. However, it should be emphasized that all the above-mentioned structures often appeared within the same melanosome (Fig. 3). Both the degree of melanization as well as the manner whereby the melanization took place varied greatly within the melanosomes (Fig. 4). This additionally contributed to the variations in the morphology of the melanosome. The position of the melanosomes in the cyto-

plasm was quite random in relation to the degree of melanization, in contrast to what is seen in the benign melanocyte. The number of melanosomes in the cells varied greatly, presumably dependent on the biological cycle of the individual cell. Thus the morphology of the melanosomes in the malignant cell varied greatly and many structural forms were found within the same cell. A close examination of all melanosomes did, however, permit the identification of cells that seemed similar, viz. belonged

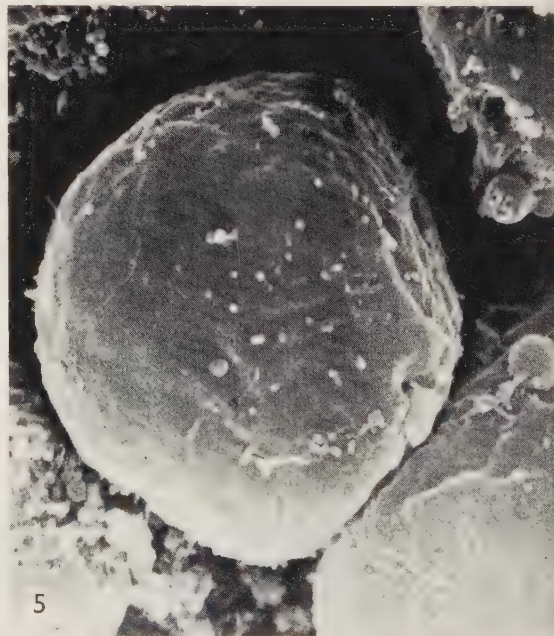


Fig. 5. Malignant melanoma cell with smooth surface. SEM, $\times 6260$.

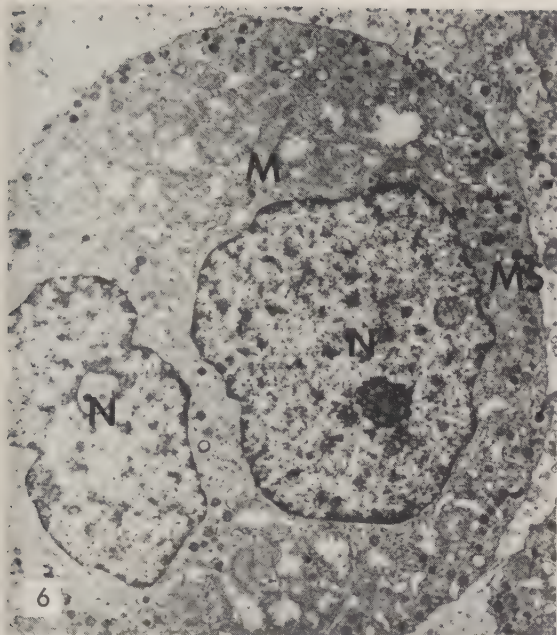


Fig. 6. Malignant melanoma cell from the same tumour biopsy as in Fig. 5. The cell surface is smooth. Two nuclei (N), numerous melanosomes (MS), and hypertrophied mitochondria (M) can be seen. TEM, $\times 4350$.



Fig. 7. Malignant melanoma cell with uneven surface, covered with microvilli (mV) and microblebs (mB). SEM, $\times 6120$.

to the same clone (cell line). Within the same tumour, 2 or 3 different cell clones often appeared.

On the basis of the above studies on the appearance of the melanosomes in numerous cells it was not possible to employ the structure of the melanosome in the differentiation between SSM and NM. In 4 patients it was possible to investigate both primary tumour and metastases in the dermis and/or in the lymph nodes, respectively. It was found that the metastases were morphologically built up of the same tumour cells or cell clones as were found in the primary tumour.

Evaluation of cytoskeleton and surface morphology of the cell in TEM and SEM

The malignant melanomas are constructed of cells with either a smooth surface (Figs. 5, 6) or an irregular, strongly folded surface (Figs. 7, 8). There are four basal surface structures: retraction fibres, microvilli, blebs, and microblebs (Figs. 15, 17, 19). These structures are distributed on the surface of the melanoma cells without any particular pattern. Within the same tumour there are cells with both smooth and folded surfaces. However, one gains

the impression that cells with a surface filled with retraction fibres, microvilli, blebs and microblebs occur mainly in ulcerated parts of tumour as well as at places where invasion of the vessel, dermis and epidermis takes place by means of individual invading cells. Moreover, on the cell surfaces, presumably at the division stage, there are always many projections (Fig. 9). Cells lying in large compact tumour infiltrates, and in nests of cells, have mainly smooth surfaces and many developed dendrites (Figs. 10, 11).

We have attempted to correlate the structure of the cell surface to the appearance of the cytoskeleton (microtubuli and microfilament system) by investigating cells with TEM and SEM within the same tumour region. The cytoskeleton in malignant melanoma cells is extremely difficult to evaluate as the inner cell is often filled with organelles such as mitochondria melanosomes and Golgi vesicles. These organelles partially cover microtubuli and microfilaments and complicate observation of the system as a whole. *It should be emphasized that microtubuli are to be found in practically all malignant melanoma cells.* Many of these

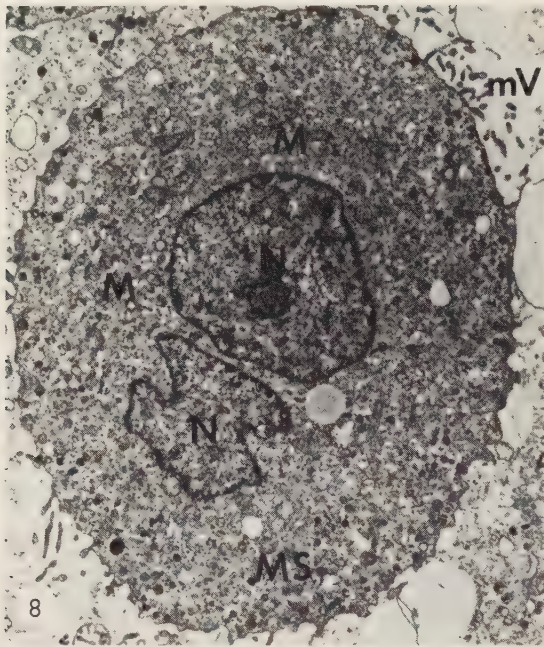


Fig. 8. Malignant melanoma cell from the same tumour biopsy as in Fig. 7. The cell membrane is uneven with numerous microvilli (*mV*). Two nuclei (*N*), numerous melanosomes (*MS*), and mitochondria (*M*) can be seen. TEM, $\times 4250$.

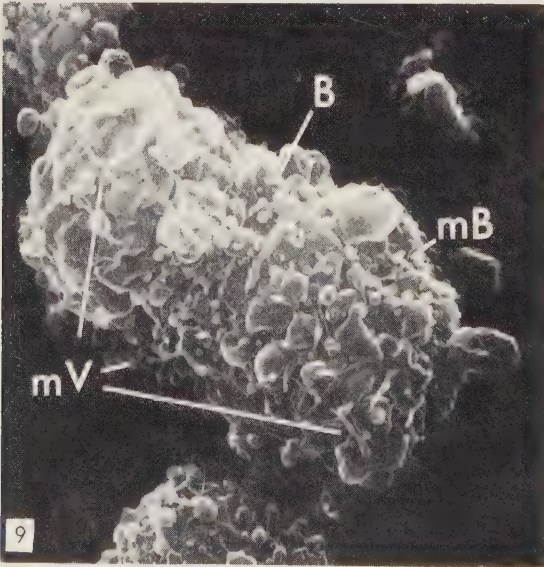


Fig. 9. Malignant melanoma cell, probably undergoing division with formation of daughter cell. The surface is uneven with many blebs (*B*), microblebs (*mB*), and a few microvilli (*mV*). SEM, $\times 4560$.

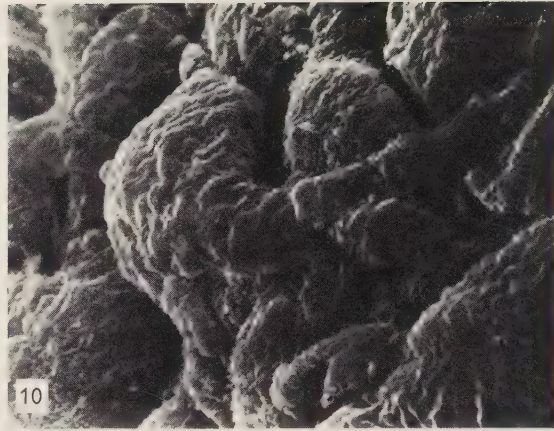


Fig. 10. Dendritic malignant melanoma cell. The cell surface is relatively smooth. SEM, $\times 4440$.

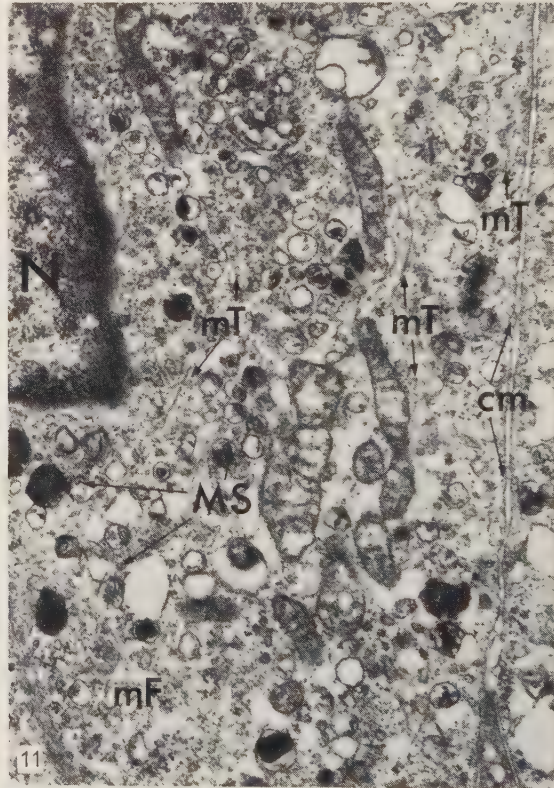


Fig. 11. Part of the malignant melanoma cell from the same tumour biopsy as in Fig. 10. Nucleus (*N*) and abortive melanosomes (*MS*) can be seen. The cell membrane (*CM*) is smooth. There are rather many microtubuli (*mT*) which course more or less parallel with the cell membrane. Microfilaments are scattered in the cytoplasm. TEM, $\times 15550$.

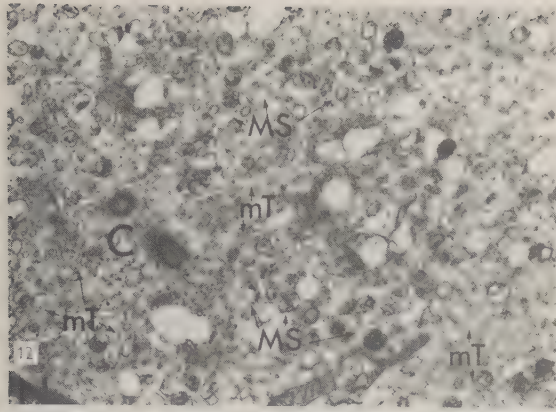


Fig. 12. Part of malignant melanoma cell. Nucleus (N), abortive melanosomes (MS), two centrioles (C), and many microtubuli (mT) can be seen. TEM, $\times 11\,900$.

cells have an apparently well-functioning microtubulus system, compared with normal melanocytes from the same patient (Figs. 12, 13). Despite a thorough examination of several sections from numerous malignant melanoma cells and tumours it has not been possible to observe any distinct pattern for the course of the microtubuli as far as direction and position are concerned that could be used as a characteristic for these malignant cells. However, in a few cells the microtubuli were found to be intertwined and collected at one site in the cytoplasm (Fig. 14). These occasional findings could not, however, be correlated to the tumour type, clinical course, or to the surface morphology. In some cells with a smooth surface (Fig. 10) micro-

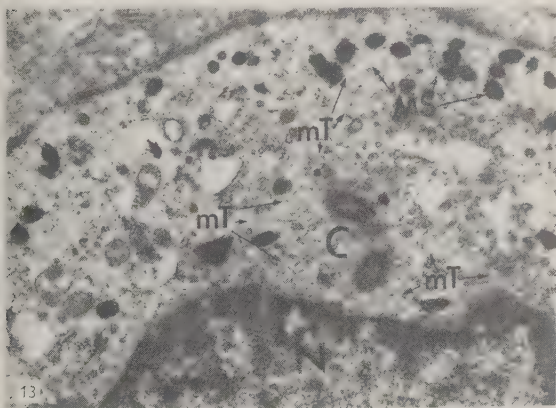


Fig. 13. Part of the melanocyte. Biopsy of normal skin from the same patient as in Fig. 12. Nucleus (N), fully melanized melanosomes (MS), two centrioles (C), and many microtubuli (mT) can be seen. TEM, $\times 11\,900$.

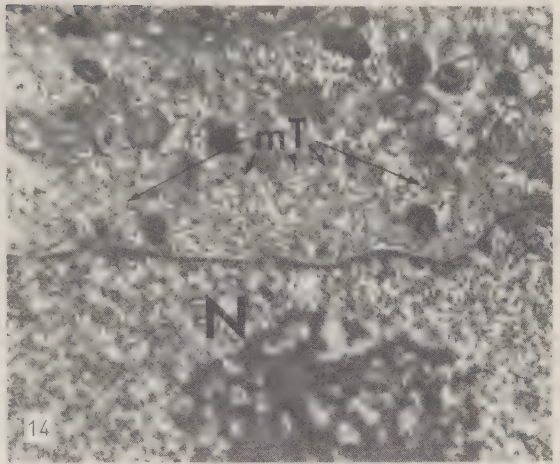


Fig. 14. Part of a malignant melanoma cell with numerous intertwined microtubuli (mT), gathered in a small area of the cytoplasm near the nucleus (N). TEM, $\times 15\,975$.

tubuli were found coursing parallel with the surface (Figs. 11, 16). Other cells with the surface filled with microblebs and microvilli (Figs. 17, 19, 24) had microtubuli running out radially in relation to the surface. Occasionally these microtubuli were observed in villi and microbleb formations (Figs. 18, 20, 25). Moreover, the blebs contained either amorphous substance or melanosomes (Fig. 20).

Microfilaments are found in varying quantities in the cells. In the cells with many microfilaments, these latter were arranged in intertwined bunches (Fig. 21) in contrast to normal melanocytes which have many microfilaments arranged in regular bundles lying parallel with the nucleus, cell mem-

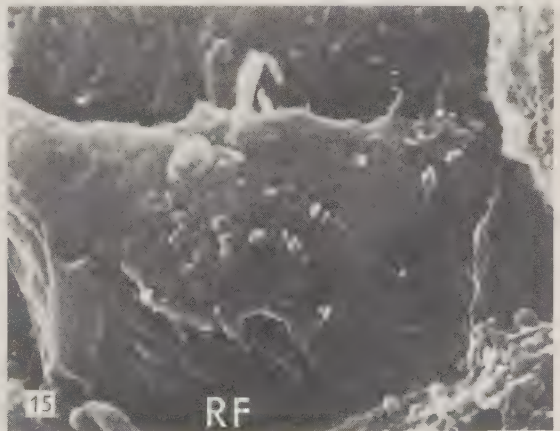


Fig. 15. Malignant melanoma cell. Part of the cell surface is even, another part uneven with many retraction fibres (RF). SEM, $\times 7\,400$.

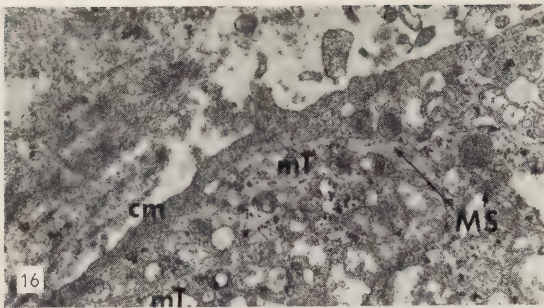


Fig. 16. Part of a malignant melanoma cell from the same tumour biopsy as in Fig. 15. The cell membrane is relatively even. Microtubuli (*mT*) course parallel with the cell membrane. Some abortive melanosomes (*MS*) can be seen. TEM, $\times 13\,850$.

brane and dendrites (Fig. 22). In several malignant cells large microfilament complexes were found collected in the shape of an island in the cytoplasm (Fig. 23). In some cells with irregular surfaces the microfilaments intertwined beneath the cell membrane (Figs. 20, 25). In other cells with smooth surfaces the microfilaments coursed parallel with the surface. In cells with few microfilaments these latter had the appearance of being loosely distributed in the cytoplasm.

DISCUSSION

Clark et al. (1972) have demonstrated that malignant melanoma cells can be divided into four types

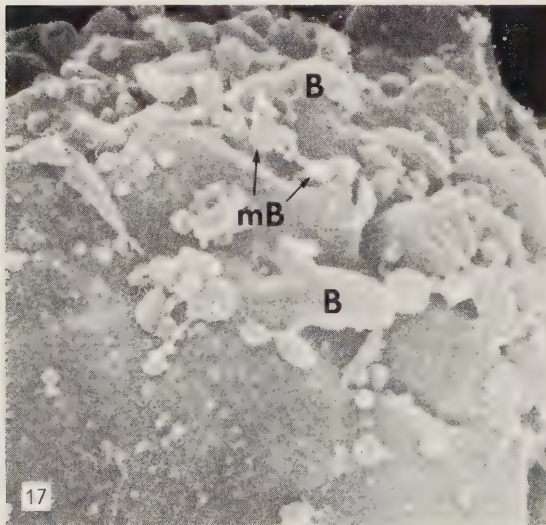


Fig. 17. Part of a malignant melanoma cell with irregular surface caused by blebs (*B*) and microblebs (*mB*). SEM, $\times 10\,800$.

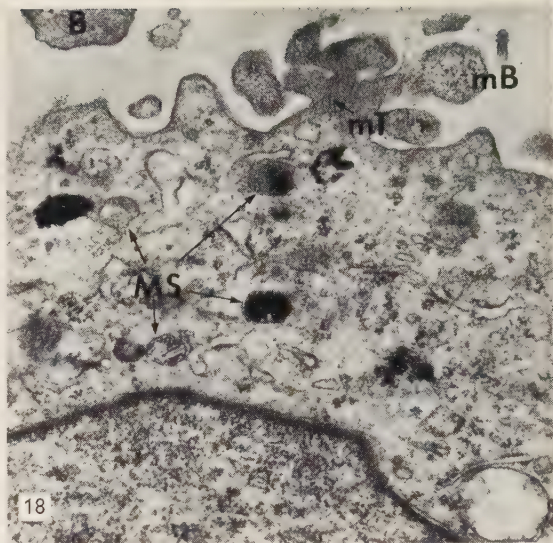


Fig. 18. Part of a malignant melanoma cell from the same tumour biopsy as Fig. 17. Cell membrane is uneven, forming blebs (*B*) and microblebs (*mB*). In one of the microblebs, a microtubulus (*mT*) can be seen. In the cytoplasm there are many abortive melanosomes (*MS*). TEM, $\times 15\,300$.

on the basis of nucleus, melanosome and mitochondria morphology. After a thorough examination of the entire material it was not possible to use Clark's classification, since only highly differentiat-



Fig. 19. Malignant melanoma cells. Cell at the bottom of the figure has a heavily folded surface, caused by numerous blebs (*B*), microblebs (*mB*) and a few microvilli (*mV*). SEM, $\times 4\,600$.

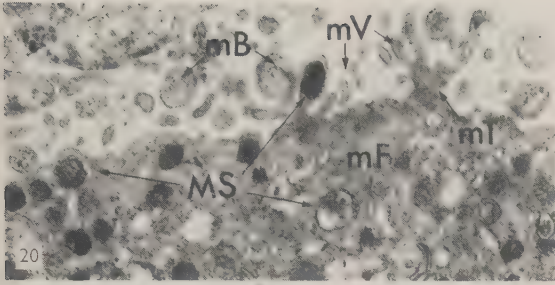


Fig. 20. Part of a malignant melanoma cell from the same tumour biopsy as in Fig. 19. Cell membrane is heavily folded, forming microblebs (*mB*) and microvilli (*mV*). Beneath the cell membrane and in the microvilli one can see intertwined microfilaments (*mF*) and some microtubuli (*mT*). Many melanosomes (*MS*) can be seen in the cytoplasm; some of them are apparently in the microblebs (*mB*). TEM, $\times 13\,200$.

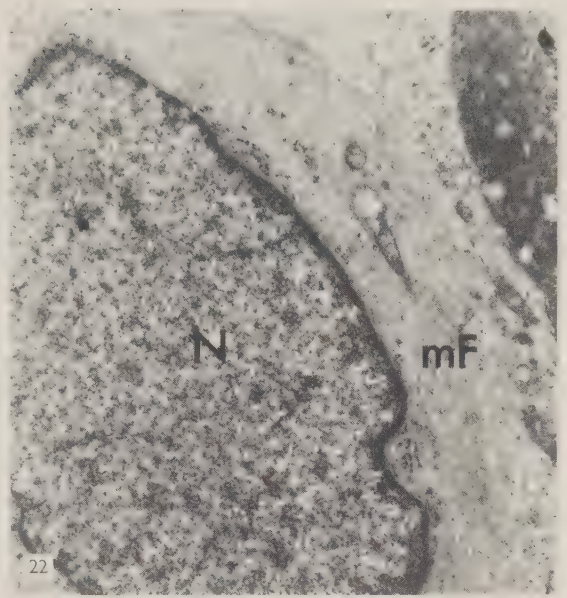


Fig. 22. Part of a melanocyte with many microfilaments (*mF*). These microfilaments are close to the nucleus (*N*); some of them radiate from the nucleus. Microfilaments are coursing in ordered bundles parallel with the nucleus and cell membrane. TEM, $\times 13\,420$.

ed cells with almost normal, hypertrophic, melanosomes, cells of type I, fulfilled their criteria. However, we have only investigated the invasive nodule of the tumours. Thus it is possible that other, more specific cell types are to be found, chiefly in the periphery of the primary tumour. Hunter et al. (1976) found no difference in the morphology of the melanosomes in invasive nodules of SSM, NM and in metastases. Our study confirms their work. Neither have we been able to ascertain any differ-

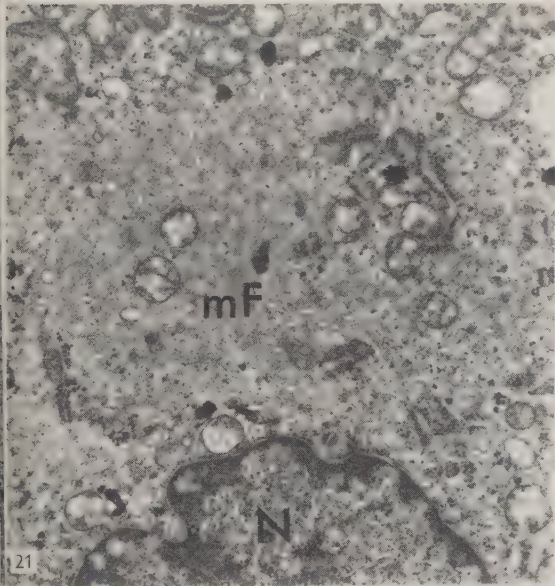


Fig. 21. Part of a malignant melanoma cell with many microfilaments (*mF*). Microfilaments are arranged mainly in bundles which are heavily intertwined and run at random. TEM, $\times 13\,420$.

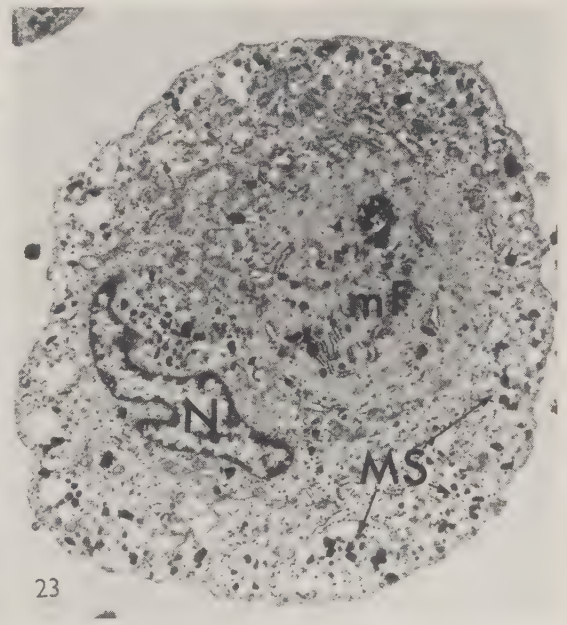


Fig. 23. Malignant melanoma cell. A big patch of intertwined microfilaments (*mF*) is seen in the cytoplasm at some distance from the nucleus (*N*). Melanosomes (*MS*). TEM, $\times 4\,300$.

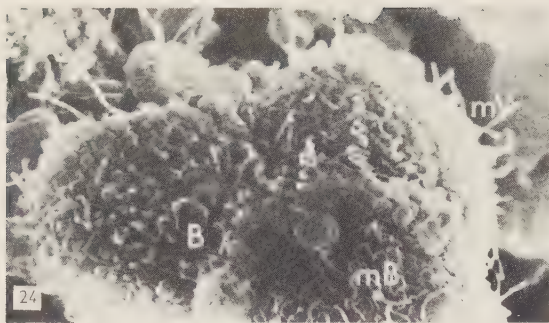


Fig. 24. Malignant melanoma cells. Many blebs (B), microblebs (mB), and microvilli (mV) can be seen on the surfaces of these cells. SEM, $\times 7430$.

ence between SSM and NM in the morphology of the melanosome. In 5 of the 8 NM, cells with multilobular nuclei were found which could indicate an increased activity. Moreover, we were not able to correlate the degree of differentiation of the cells on

the basis of the appearance of the melanosomes together with the clinical course.

We can confirm Curran & McCann's conclusion that malignant melanoma cells can be identified solely on the basis of the structure of the melanosomes. The morphology of the melanosomes in the melanoma cells varies greatly, probably dependent on the degree of differentiation and the biological cycle of the cell. Many structural forms of melanosomes are to be found within the same cell. However, it should be emphasized that by a thorough examination of countless melanosomes it was possible to identify cells belonging to the same cell clone. In many tumours 2–3 cell clones were to be found. Thus we are able to state, in agreement with Clark et al. (1975) that in malignant melanoma intralesional transformation often occurs, resulting in the commencement of several cell lines within the same tumour: these cell clones are variously differentiated. Meyer & Weiss (1977) found that cells

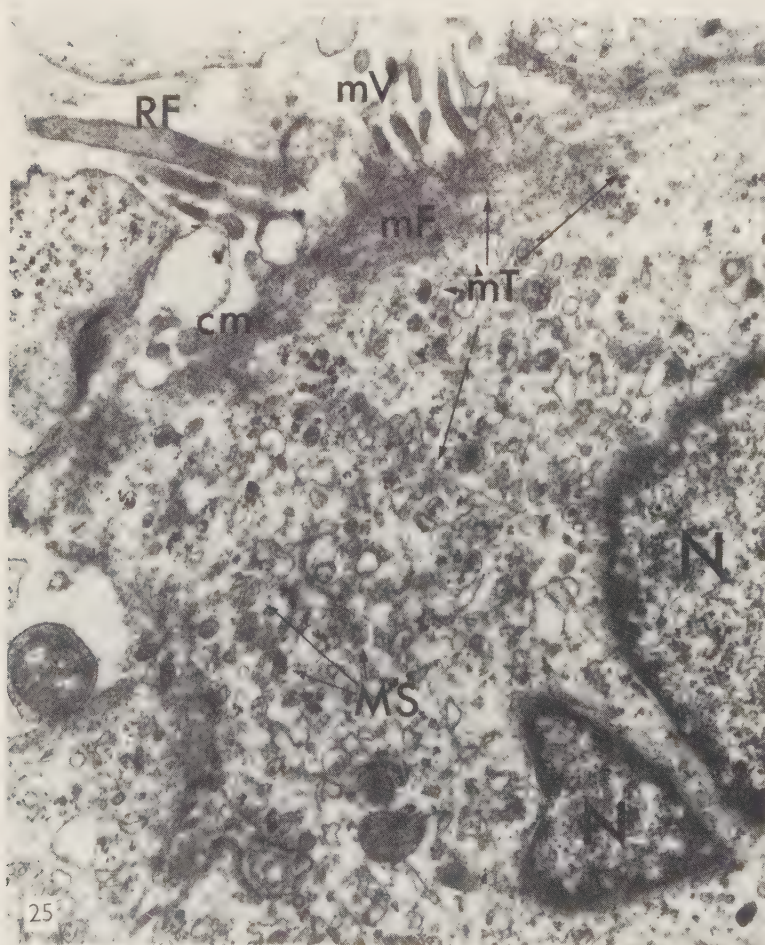


Fig. 25. Part of a malignant melanoma cell from the same tumour biopsy as in Fig. 24. Cell membrane (cm) is uneven, forming retraction fibres (RF) and microvilli (mV). Beneath the cell membrane one can see a few microtubuli (mT), many microfilaments (mF) and microfilamentous material. TEMX.

from primary malignant melanoma and metastases differentiated in different ways in cell cultures as transformation occurred in cells cultivated from metastases which resulted in the creation of several cell lines. The authors concluded that there is possibly a "relationship between these morphological alterations and the ability of the melanoma cells to produce metastases". In contrast to these observations on cell cultures we have noted that all investigated metastases *in situ* were constructed morphologically of the same cells or cell clones as were found in the primary tumour. Hence it must be concluded that malignant melanoma cells are able to preserve their phenotype despite the influence of other cells in dermis, blood vessels, lymph vessels and lymph nodes. The appearance and surface morphology of the malignant melanoma cell varies (Drzewiecki). Oval, round and dendritic cell forms with smooth or strongly folded surfaces occur. Attempts have been made to correlate these surface alterations with the appearance of the cytoskeleton (microtubuli and microfilament-complexes).

Studies by Fuller & Brinkley (1976), Miller, Fuseler & Brinkley (1977) carried out on cell cultures show that microtubuli complexes are greatly reduced after transformation of normal cells to malignant cells, and this can lead to "the increased capacity of transformed cells for *in vitro* growth". On the other hand, Lantos (1977) found that poorly differentiated neoplastic astrocytes showed many microtubules and in moderately differentiated cells there was a decrease in the number of microtubules. Osborn & Weber (1977) have also demonstrated microtubular networks in transformed cells.

Our study shows that in many malignant cells from biopsies of primary tumour and metastases, microtubuli complexes are to be found. There were either more or less in relation to normal melanocytes in the same patient. In many cells they were, like in normal melanocytes, well-built and placed in the cytoplasm. In some cells with irregular surfaces the microtubuli were arranged in another way compared with cells having smooth surfaces. But whether this is primary or secondary in relationship to the surface alterations cannot be determined with certainty. It can only be ascertained that the cytoplasmic microtubuli system participates in the formation of different surface structures such as blebs, microvilli and retraction fibres. Microvilli might play a part as the cell's contact organelle as

microvilli are often observed in contact with other cells. In certain cases microbleb formations can be a form of excretion of melanosomes from malignant cells. It should be stressed that with regard to microtubuli, research into the system as a whole in malignant melanoma cells is complicated by an accumulation of countless cell organelles. Moreover the microtubuli system probably frequently and rapidly changes its morphology, depending on the biological cycle of the cells—which can differ widely within the same tumour. Thus a static investigation method, such as TEM, does not provide great possibilities for investigating the whole system in depth. Despite these reservations the microtubuli system was found to be well built in many cells; though in some cells there were clear signs showing that it was functioning poorly. All in all, the appearance and quantity of the microtubuli cannot be used as a definite sign of the malignancy of that cell. With regard to microfilaments, Kanazaki, Hashimoto & Bath (1977) found large quantity of microfilaments in malignant melanoma cells and concluded that the "amount of actin-like microfilaments in melanocytic cells is inversely correlated to the differentiation of the cells *in vitro* and *in vivo*".

In our study we found that the quantity of microfilaments in malignant cells can either be increased or considerably reduced compared with normal melanocytes in the same patient. Three of the melanomas that metastasized had large quantities of microfilaments, and these were placed abnormally. Possibly this is related to the biological behaviour of the tumour.

Moreover, it was found that in many cells the microfilaments were apparently functioning poorly inasmuch as when they occurred in large numbers they are arranged in intertwined and irregular bunches; in smaller quantities they occur scattered in the cytoplasm in contrast to normal melanocytes (Jimbow, Pathak & Fitzpatrick, 1973; Jimbow & Fitzpatrick, 1975). This lack of 'arrangement' is possibly of importance for the malignant behaviour of the cell, for Felix & Sträuli (1976) have shown that resting leukaemia cells have microfilaments arranged regularly in large bundles and on commencing movement these filaments become loosely distributed in the cytoplasm.

A poorly functioning microfilament system can be of importance for the antigen behaviour of the cell, for Albertini & Anderson (1977) have demon-

strated that well-functioning microfilament complexes participate actively in the process of capping of Con A receptors on the surface of cultured granulosa cells.

The clinical course of the malignant melanoma in our material can best be correlated with the histogenetic tumour type, the depth of invasion, the shape of the nucleus in the cells and the quantity and arrangement of the microfilament complexes. With regard to surface morphology, there was no relation between this and the type and clinical course of the tumour. However, in several cases it was found that the apparently invading cells had strongly folded surfaces that are conceivably related to the behaviour of the cell.

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THE EPIDERMAL MELANOCYTE SYSTEM IN PATIENTS WITH MALIGNANT MELANOMA¹

Quantitative and Qualitative Investigation of Dopa Positive Melanocytes

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Abstract. The results from this study indicate that in 5 out of 22 patients increased activity of the melanocytes in the vicinity (a few mm to 1 cm) of the tumour is to be found. In 8 out of 22 patients suspect cells were also found a few mm to 1 cm from the tumour; a finding which accounts for the frequent recurrence following close excision of the melanoma. Furthermore, it was ascertained that the total melanocyte population is increased in 14 out of 22 patients. An increased melanocyte population can contain a greater number of instabile cell forms that can easier be malignantly transformed under the influence of external factors.

Recent melanoma research has shown that the histogenetic type and the size of the tumour, the depth of the invasion and the anatomical region in which the tumour appears are decisive factors for the course of the disease.

A result of the above is that different centres recommend varying excision distances for primary melanomas dependent on these factors. That is to say, the less malignant tumour requires close excision, while the highly malignant tumour demands wider excision (Davis & McLeod, 1972; Harris & Gumport, 1976). The rational local treatment of the malignant melanoma should be based on the primary pathogenesis, growth and spread pattern of the tumour. According to Davis (1976) two theories concerning the origin and growth of the melanoma have been proposed though not proved. According to one of these theories the tumour arises as a consequence of malignant transformation of melanocytes in a specific area and the tumour grows by simple cell division. The second theory proposes that the tumour originates simultaneously in large or small fields of skin and grows not only by

cell division but also by malignant transformation of adjacent normal melanocytes. Willis (1967) supports this second theory.

As far as the spread pattern of the tumour is concerned, Petersen, Bodenham & Lloyd (1962) and Olsen (1966) have propounded the theory that local spread of the malignant melanoma occurs by transference of malignant potential from tumour cells to surrounding normal melanocytes, which are both activated and also directly transformed malignantly. It has been proposed that this transference takes place through the dendrites. Weiss (1976) is of the opinion that some metastases could arise by the freeing of sub-cellular particles from tumour cells to the surroundings.

Observations supporting these spreading theories have been made by, among others, Cochran (1971) who has found junctional field changes of melanocytes surrounding the superficially spreading melanomas and lentigo malignant melanomas. McGovern (1972) has stated that significance of melanoma with a spreading in-situ phase is that it represents a field effect and skin for variable distance around the initial lesion has a melanoma-producing potential. Also Seiji & Takahashi (1974) have described melanoma forms with adjacent intraepidermal proliferation of melanocytes. Finally, Wong (1970) has observed an increased melanocyte population at a distance of 5 cm from the tumour in 7 out of 12 patients with primary skin melanomas.

The extent of the above-mentioned changes in the melanocyte population surrounding malignant

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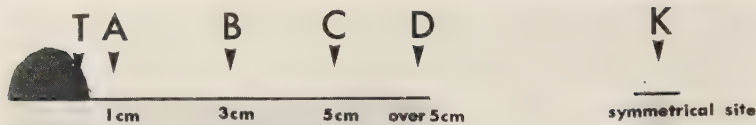


Fig. 1. Schematic presentation of the biopsy specimens taken from each patient.

melanoma should be decisive for the planning of local surgical treatment of the tumour.

The objective of this present study was the quantitative and qualitative evaluation of the melanocytes surrounding malignant melanoma compared with those at an anatomically symmetrical site on the same patient, and with the distribution of melanocytes in normal healthy individuals (Staricco & Pinkus, 1957; Mitchell, 1963; Szabo, 1967).

MATERIAL AND METHODS

The investigation comprised 22 patients of Scandinavian descent. 18 had typical skin melanomas; of these 13 were classified as superficial spreading melanomas (SSM) and 5 as nodular melanomas (NM). One patient had 2 primary melanomas, both tumours being classified as SSM. In one patient the tumour disappeared leaving a vitiliginous area as well as cutaneous metastases constructed of amelanotic tumour cells. In one patient the tumour was in the process of disappearing into a larger vitiliginous area. This tumour had metastasized to the regional lymph nodes. One patient had a local cicatrix after close removal of the tumour in

addition to metastases to the regional lymph nodes. The primary tumour had been excised some months prior to the radical operation.

No pathological condition was found in any of the above patients capable of producing alterations in the skin pigmentation. Patients who were operated in the summer months were slightly sun-tanned.

All tumours were excised at a minimum distance of 5 cm, apart from SSM on the face which was removed at a distance of 2 cm.

From each excision specimen 5 skin biopsies were obtained each measuring approximately 5×10 mm. The biopsies were excised at distances of 0 cm, 1 cm, 3 cm, 5 cm and more than 5 cm from the tumour and this sequence was labelled T, A, B, C, D, respectively (Fig. 1). All biopsies were obtained in a line from the tumour to the regional lymph nodes. Provided the site of the tumour permitted, a control biopsy, labelled K, was obtained from an anatomically symmetrical site.

In 2 patients a series of biopsies was obtained from the edge of the tumour out to the line of excision so that a whole strip of skin has been included in the microscopic investigation.

The skin biopsies were incubated in a solution of 1.0–2.0 N NaBr for about 3 h. Then the epidermis was separated from the dermis under a dissection microscope. The separated epidermal sheets were stained with DOPA

Table I. Malignant melanoma of the lower leg. Summary of the investigation of the epidermal melanocytes population

Tumour type	Case no.	Melanocyte count per square mm range (mean)	Statistical analysis ^a	Comparison of the melanocyte count with other materials ^b	Increased activity of the melanocytes in vicinity of the tumour	Abnormal cell forms in vicinity of the tumour
SSM	X	735–1 800 (1 283)	K=A<B=C=D	=		
SSM	XI	734–2 057 (1 350)	$p>0.01$	=	+	
SSM	XV	1 414–3 003 (2 082)	K=A=T≤B<C=D	>	+	+
Scar	XVI	1 634–3 397 (2 599)	$p>0.01$	>		
SSM	XVII	1 836–2 966 (2 307)	$p>0.01$	>	+	
SSM	XVIII	3 021–5 812 (4 022)	T=B=C=D<K	>		
tumour+vitiligo	XIX	384–2 433 (704)	T=A<B=C=D=K	<		
SSM	XX	456–1 680 (1 081)	$p>0.01$	=		
no						
tumour+vitiligo	XXIII	496–1 910 (1 150)	$p>0.01$	=		
SSM	XXVII	1 377–3 030 (2 063)	$p>0.01$	>		
SSM	XXIX	330–3 232 (2 002)	$p>0.01$	>		+
NM+vitiligo	XXXI	487–4 398 (2 639)	T=B<C=D=K	>		

^a Statistical analysis of the melanocyte count between biopsy specimens within the same patient (one way analysis of variance).
^b Number of the melanocytes in the epidermis of normal adults, given by: Mitchell 310–1 670 (1 000), Staricco & Pinkus 500–1 440 (1 031), Szabo 1 510±170 (SE).

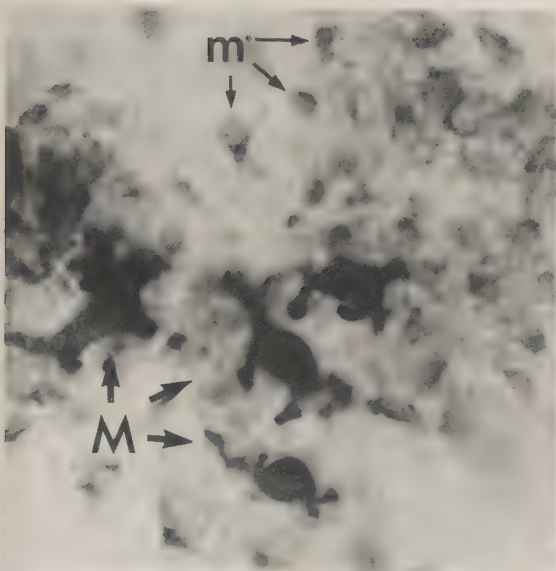


Fig. 2. A group of darkly stained, giant malignant cells (M), surrounded by normal melanocytes (m). Epidermal preparation and DOPA treatment of the skin taken near the edge of the malignant melanoma of the back. ($\times 350$.)

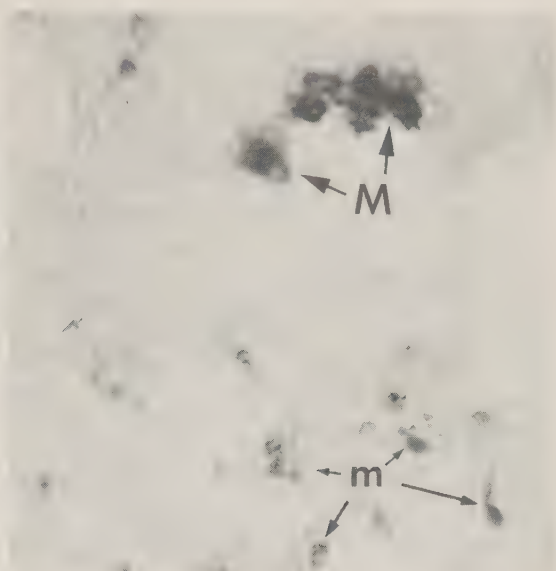


Fig. 3. A group of darkly stained, round, abnormal melanocytes (M), which are much bigger than normal melanocytes (m), and do not form dendrites. Epidermal preparation and DOPA treatment of the skin taken from the vicinity of the malignant melanoma of the back. ($\times 120$.)

(Staricco & Pinkus). After collection of the materials, blind evaluation of the number of melanocytes and their morphology was undertaken in all preparations. The melanocytes were counted in 4–10 areas, each 0.1089 mm², chosen at random, using a graticule fitted in the eyepiece of the microscope, and at a magnification of $\times 400$. The melanocyte total was expressed as the total of cells per mm² of the epithelial surface.

Among other things in the morphological evaluation, attention was paid to the density and number of the papillae for it is to be assumed that in strongly developed epithelium, it should be possible in a determined area to count more cells than in a weakly developed epithelium.

Attention was also paid to the morphology of the melanocytes, the intensity of the DOPA staining as well as to the distribution of the cells that can vary. In each patient an average of about 5000 to 12000 cells were investigated.

One-way analysis of the variance was employed in the statistical treatment of the material.

RESULTS

The epidermal melanocyte population in normal individuals varies depending on the anatomical region. In the present material the patients have been distributed according to the regional siting of the melanoma.

Patients with primary tumour of the lower leg. This group comprised 12 patients (Table I). In 7 of them it was not possible to demonstrate statistical

differences between the melanocyte totals for the biopsies labelled T, A, B, C, D, and K, respectively. In the remaining 5 patients a significant difference was found ($p < 0.01$). Patients X and XV had more melanocytes at a greater distance from the tumour than close to it. The number of cells in close proximity to the tumour in these two patients was equal to that found in the control biopsy. Patient XVIII had more melanocytes in the control biopsy than in the biopsies from the proximity of the tumour. Patients XIX and XXXI had fewer cells in the immediate vicinity of the tumour than in the other locations. Both patients developed vitiliginous areas around the tumour.

In 2 out of 12 patients suspect cells were located from a few mm up to 1 cm from the tumour. These cells were seen either as large, dark-stained dendritic cells similar to malignant cells as seen in the biopsies from the tumour, or as large, oval, adenodendritic, strongly-pigmented cell forms (Figs. 2, 3).

In 3 out of the 12 patients with melanomas of the lower leg, an increased activity of all melanocytes in the vicinity of the tumour was observed. This activity comprised a zone of a few mm to up to 1 cm from the edge of the tumour. With DOPA staining

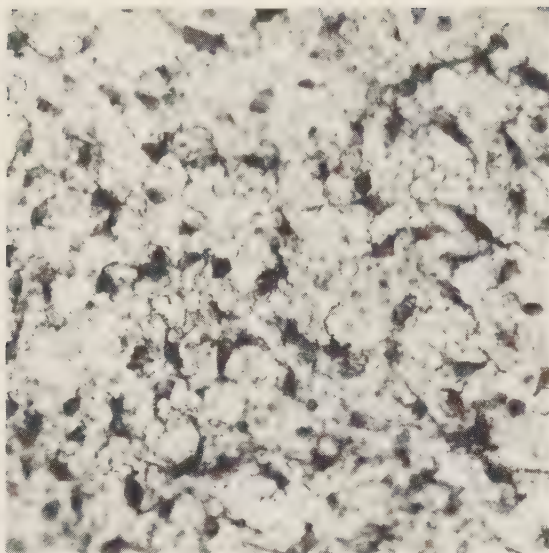


Fig. 4. Darkly stained, hypertrophied, dendritic melanocytes. Epidermal preparation and DOPA treatment of the skin taken from the vicinity of the malignant melanoma of the lower leg. ($\times 118$.)

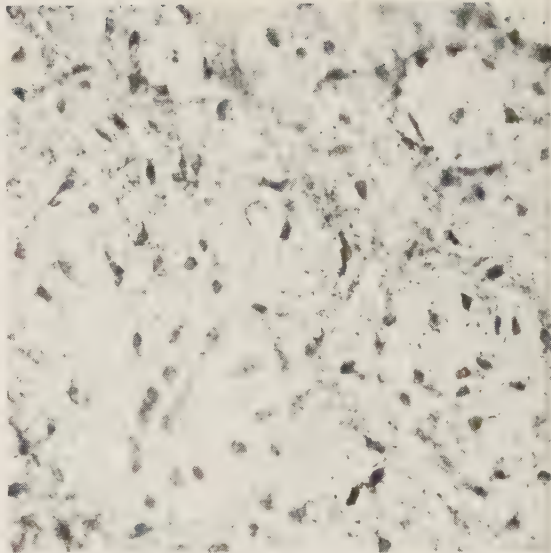


Fig. 5. Normal melanocytes. Epidermal preparation and DOPA treatment of the skin taken from the lower leg. A symmetrical site to biopsy specimen presented in Fig. 4. ($\times 118$.)

these melanocytes are strongly brown-black in colour, they are larger than unactivated cells and develop several multi-branched dendrites (Fig. 4). The number of cells is not increased in relation to the number of melanocytes at an anatomically symmetrical site (Fig. 5).

In all preparations a strong cell polymorphy was observed in addition to irregular DOPA staining that could indicate a cyclic function of the melanocyte.

Comparison of the melanocyte populations in all biopsies in each investigated patient with that found on the lower leg of normal healthy individuals (Table I) shows that 7 out of the 12 patients had a considerably greater number of melanocytes per mm^2 , while one patient with vitiligo had fewer melanocytes than in the normal material. The configuration of the dermal side of the epidermis had no influence on this data.

Patients with primary tumour of the back. There were 8 patients in this group (Table II). In 5 of them no significant difference could be demonstrated between the number of melanocytes in the biopsies labelled T, A, B, C, D, and K. In the 3 remaining patients a significant difference $p < 0.01$ was found. Patients XIV and XXVI had more melanocytes surrounding the tumour than in the control biopsy. Patient no. XXX had considerably fewer melano-

cytes in the biopsy labelled C compared with the remaining biopsies.

An increased melanocyte activity in the vicinity of the tumour was not observed in any of the patients. In 4 out of the 8 patients suspect cells were observed up to 1 cm from the edge of the tumour. In one of these (patient XIII a) the abnormal cells were seen in all biopsies except the control biopsy K. In all of the 8 patients of this group predominantly oval or polydendritic cell shapes were seen. The intensity of the DOPA staining varied greatly.

The melanocyte population in all biopsies was considerably increased in 5 out of the 8 patients compared with that on the back of normal adults (Table II). It was not possible here, either, to find a relation between this data and the influence of the sun or the configuration of the dermal side of the epidermis.

Patients with primary melanoma of the abdomen. This present investigation included only two patients in this category (Table III). In one of them (patient XIII b) the number of melanocytes in biopsies varied significantly $p < 0.01$. This patient presented minor melanocyte activity in the immediate vicinity of the tumour. No difference was found between the melanocyte count in the remaining patient's biopsies, however, suspect cells were found in close relation to the tumour.

Table II. *Malignant melanoma of the back. Summary of the investigation of the epidermal melanocytes population*

Tumour type	Case no.	Melanocyte count per square mm range (mean)	Statistical analysis ^a	Comparison of the melanocyte count with other materials ^b	Increased activity of the melanocytes in vicinity of the tumour	Abnormal cell forms in vicinity of the tumour
NM	XIV	1 533-3 398 (2 518)	T=A=B=C=D>K	>		+
NM	XXI	569-1 919 (1 184)	$p>0.01$	=		+
SSM	XXII	551-2 764 (1 276)	$p>0.01$	=		
SSM	XXIV	872-2 755 (1 831)	$p>0.01$	>		
NM	XXVI	357-2 819 (1 406)	K=A=B<C=D	=		
			T>D, CA, B, K			
SSM	XXVIII	2 755-5 335 (3 977)	$p>0.01$	>		
NM	XXX	211-3 471 (2 006)	A=B=D=K>C	>		+

^a Statistical analysis of the melanocyte count between biopsy specimens within the same patient (one way analysis of variance).

^b Number of the melanocytes in the epidermis of normal adults, given by: Mitchell 940-1 990 (1 360), Staricco & Pinkus 1 290-1 390 (1 340), Szabo 930 ± 140 (SE).

Both patients had considerably more melanocytes in all biopsies compared with the number of melanocytes in normal adults (Table III). Also here the configuration of the dermal side of the epidermis was without influence on this data. Both patients had melanomas on sun-protected areas of the skin.

Primary tumour localized to the face. No difference was found between the melanocyte populations of the various biopsies. The total number of the melanocytes was slightly increased. The presence of the tumour activated surrounding melanocytes. Among the activated cells suspect cells were also found (Table IV).

DISCUSSION

In 5 out of 22 patients with cutaneous malignant melanomas activation of the melanocytes in the vi-

cinity of the tumour (a few mm to 1 cm) was observed. All five patients had superficially spreading melanomas. In patients with nodular melanomas no activation of the melanocytes surrounding the tumour was to be found. Cochran has demonstrated that the activation of the melanocytes surrounding the tumour can be unidirectional, while cells in the remainder of the circumference remain inactivated. As the biopsies in this present investigation represent a single part of the adjacent epithelium it is to be assumed that several areas with activated melanocytes could be found by investigating the epithelium circularly around the tumour.

On the basis of the present investigation it is not possible to comment on the mechanism of this activation. Bodenham (1968) has ascertained that the skin temperature above the malignant melanoma is increased. As increased temperature in certain

Table III. *Malignant melanoma of the abdomen. Summary of the investigation of the epidermal melanocytes population*

Tumour type	Case no.	Melanocyte count per square mm range (mean)	Statistical analysis ^a	Comparison of the melanocyte count with other materials ^b	Increased activity of the melanocytes in vicinity of the tumour	Abnormal cell forms in vicinity of the tumour
SSM	XIIIb	2 635-5 078 (3 641)	$p<0.01$	>	+	
SSM	XXV	1 414-3 673 (2 824)	$p>0.01$	>		+

^a Statistical analysis of the melanocyte count between biopsy specimens within the same patient (one way analysis of variance).

^b Number of the melanocytes in the epidermis of normal adults, given by: Mitchell 500-1 320 (800), Staricco & Pinkus 400-1 270 (754), Szabo 800 ± 40 (SE).

Table IV. *Malignant melanoma of the cheek. Summary of the investigation of the epidermal melanocytes population*

Tumour type	Case no	Melanocyte count per square mm range (mean)	statistical analysis ^a	Comparison of the melanocyte count with other materials ^b	Increased activity of the melanocytes in vicinity of the tumour	Abnormal cell forms in vicinity of the tumour
SSM	L	1 735-3 453 (2 430)	$p > 0.01$	$\geq$	+	+

^a Statistical analysis of the melanocyte count between biopsy specimens within the same patient (one way analysis of variance).

^b Number of the melanocytes in the epidermis of normal adults, given by: Mitchell 1 120-2 300 (1 570), Staricco & Pinkus 700-2 000 (1 340), Szabo $2\,310 \pm 150$ (SE).

cases is capable of activating the epidermal melanocytes (El Mofty, 1968), this can be regarded as one of the possible causes of melanocyte activation surrounding the tumour. Cochran (1969) is of the opinion that later junctional change is either a residuum of the focus of malignant transformation of the melanocytes which precedes the development of invasion or is the remains of a pre-existing junctional or compound naevus. Finally, Petersen, Bodenham & Lloyd and Olsen claim that melanocytes can be activated via dendritic links from the adjacent malignant melanoma. In 8 patients abnormal cell forms were found in the immediate vicinity of the tumour (a few mm to 1 cm). These do not appear to be related to the type of tumour nor do they appear to be related to the activation of the surrounding melanocytes. The presence of these abnormal cell forms can be interpreted as a form of horizontal spread of the tumour (Clark, From, Bernardino & Mihm, 1969; McGovern) or as a multifocal origin of the tumour (Willis). In 1 of the 8 patients (XIIIa) the suspect cells were found in all biopsies except the control one. The patient in question had multiple melanomas (Tables II; III).

The occurrence of suspect cell forms in the vicinity of the tumour fully explains the frequent recurrences following the close removal of the melanoma. Statistical analysis of the melanocyte population in all biopsies from each patient showed that in 13 out of 22 patients there was no significant difference to be found among the biopsies. Only 2 out of 22 patients had more melanocytes surrounding the tumour than in the control preparations from symmetrical sites. Thus it can be concluded that the presence of malignant melanoma is not synonymous with an increased melanocyte count in its surroundings.

Two patients who developed vitiliginous zones

had fewer melanocytes surrounding the tumour than in the other biopsies. This phenomenon can be interpreted as an expression of the immunological elimination of tumour cells and the surrounding melanocytes.

In the remaining 5 patients a significant difference was demonstrated between the melanocyte counts from several biopsies though without relation to the distance from the tumour. This probably reflects a varying distribution of the melanocytes as well as loss of some cells during the separation procedure (Drzewiecki & Kjærgaard, 1977)—both in the tumour region (ABCK) and at the control site (K).

The investigation has surprisingly shown that in 14 out of 22 patients the total melanocyte count is increased in relation to the count in normal individuals. For comparison purposes the totals of the melanocyte population in healthy adults as stated by Mitchel, Staricco & Pinkus and Szabo are used.

In an investigation of the melanocyte population at a distance of 5 cm from the tumour, Wong (1970) has observed an increase in the cell count in more than half the investigated patients, and concludes that the presence of the tumour is to be held responsible. While Wong has not investigated control biopsies from anatomically symmetrical sites it is to be presumed that he has observed the same phenomenon, viz. a total increase in the melanocyte population in patients with malignant melanoma.

More than half of the patients in this present investigation had an increased melanocyte population when compared with normal individuals.

An examination of the complete material revealed that neither the influence of the sun nor the development of the dermal side of the epidermis affected this data. The patients had no disturbances of the pigment other than the malignant melanoma. Thus it must be presumed that an increased

melanocyte population can be genetically conditioned in the investigated patients and that probably this could give rise to several instabile cell forms and thereby increase the possibility of malignant transformation by external influences. Such external influences could be the sun (Lee & Merrill, 1970) and the environment (Smart & Carle, 1975). It should be emphasized that no investigation has been undertaken of the melanocyte population in a normal Scandinavian population, and that the above conclusions are based on a comparison of the present material with data given by Staricco & Pinkus, Mitchell and Szabo for the distribution of melanocytes in normal healthy individuals.

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THE EPIDERMAL MELANOCYTES IN PATIENTS WITH CUTANEOUS MALIGNANT MELANOMA¹

A Transmission Electron Microscopic Investigation

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Abstract. The epidermal melanocytes were studied in biopsies from clinically normal skin removed at various distances from cutaneous malignant melanomas. Melanocytes with some ultrastructural changes were found in a number of patients, which can be either contingent on genetic or inter/extra cellular factors. It is possible that cells with these changes are more instable and more easily transformed to malignant cells by external influence. Malignant cells were not demonstrated in either the epidermis or the dermo-epidermal junction. Activation was seen of the epidermal melanocytes lying in the immediate neighbourhood of the superficially spreading melanomas, which would suggest that this type of tumour is presumably able to influence the melanocytes. The most pronounced changes in the whole of the melanocyte system was found in patients with multiple melanomas, and in a patient with metastasizing malignant melanoma.

tron microscopy (TEM) in the same skin biopsies earlier evaluated by means of DOPA staining (Drzewiecki 1979b).

MATERIALS AND METHODS

The investigation comprises 27 patients. Of 24 typical primary skin melanoma, 17 were classified as superficially spreading melanoma (SSM) and 7 as nodular melanoma (NM) (Table I). Of the four remaining patients, one developed vitiligo around regressing tumour. In the other patient the tumour disappeared leaving a vitiliginous area. A third patient developed, shortly after exstirpation of the primary tumour, dermal metastases in a well-defined skin area close to the skin graft. The fourth patient had a scar after close removal of the tumour some months prior to the radical operation.

Two patients had multiple melanomas. One of them, No. 4, developed a melanoma on the back of trunk and front of trunk simultaneously. The other patient, No. 15, had had the tumour removed from the right lower leg, and one year after the operation a new primary tumour developed on the left lower leg. No other pathological conditions were found in any of the patients, which could bring about changes in the skin pigmentation.

Biopsies were taken from a few mm, 1 cm, 3 cm, 5 cm and more than 5 cm from the primary tumour, the scar after primary excision (No. 27), the skin area with dermal metastases (No. 26), or the vitiliginous area (Nos. 24 and 25). Control biopsies were removed from the anatomically symmetrical site, providing the localization of the tumour permitted.

Each biopsy was fixed and prepared for HE-staining and TEM. The method of fixation and preparation for TEM microscopy has been described earlier (Drzewiecki & Kjægaard, 1977).

The following points were taken into consideration in the evaluation of the individual melanocytes during TEM:

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A theory suggesting that malignant melanoma is able to activate and possibly malignantly transform the surrounding epidermal melanocytes has been put forward by Petersen, Bodenham & Lloyd (1962) and Olsen (1966). The theory has apparently been rendered more probable by Wong (1970), Cochran (1971), Seiji & Takahashi (1974) and Paul & Germand (1975), who have demonstrated both quantitative as well as qualitative changes in the population of the DOPA-positive epidermal melanocytes around malignant melanoma. The extent of these changes can be of importance when planning the local surgical treatment of this tumour. The above constituted the basis for the histochemical and morphological studies of the epidermal melanocytes in biopsies taken from melanomas and clinically normal skin around the tumours (Drzewiecki, 1977, Drzewiecki 1979a, b). The aim of this study was to investigate the melanocytes in HE-stained sections as well as their fine structure in transmission elec-

Table 1. Clinical course of malignant melanomas, distribution according to tumour type, histological classification and anatomical region

Patient No.	Anatomical region	Type of tumour	Depth of invasion in Clark's levels	Metastazization to		Died from mal. melanoma
				Dermis	Lymph. node	
1	Lower leg	SSM	IV		+	+
2	Lower leg		II			
3	Lower leg		III			
4	Back of trunk		III			
5	Lower leg		IV		+	
6	Lower leg		III			
7	Lower leg		III			
8	Lower leg		III			
9	Back of trunk		II			
10	Back of trunk		III			
11	Front of trunk		III			
12	Lower leg		IV			
13	Back of trunk		III			
14	Lower leg		III			
15	Lower leg (R)		II			
16	Lower leg		III			
17	Face		II			
15	Lower leg (L)	NM	IV	+		
18	Back of trunk		IV			
			(pedunculate)		+	+
19	Back of trunk		(pedunculate)		+	
20	Back of trunk		IV			
			(pedunculate)		+	
21	Back of trunk		IV	+	+	+
22	Back of trunk		(pedunculate)	+	+	
23	Back of trunk		(pedunculate)		+	+
24	Lower leg		Tumour + vitiligo	+	+	+
25	Lower leg	No tumour + vitiligo		+		
26	Back of trunk	Dermal metastases		+	+	+
27	Lower leg	Scar			+	+

1. Nucleus—size, form, number of nucleoli and possible pseudoinclusions.

2. Melanosomes—form, structure, size, degree of melanization, position within the cell.

3. Endoplasmatic reticulum, ribosomes, Golgi apparatus and mitochondria—extent of development and morphology.

4. 100 Å filaments and microtubuli—quantity, pattern and position within the cell.

Following this, a comparative evaluation was carried out of the melanocytes in various biopsies taken from the same patient. Finally, photographic TEM material from all the biopsies from all the patients was compared.

RESULTS

HE-staining

In 6 of the 27 patients (Nos. 1, 2, 3, 9, 13, 25) nests of suspect "nevus cells" were found in the skin

biopsies removed from a few mm to 1 cm from the macroscopical border of the tumour. Typical dermal metastases were found in biopsies from the metastatic area in patient No. 26. The epidermis and epidermal melanocytes in this patient were normal. Patient No. 24 had considerably fewer melanocytes close to the vitiliginous area than in other skin areas. All the skin biopsies from the remaining patients were normal in respect of both the epidermis and dermis.

TEM studies

In 12 of the 27 patients (Nos. 2, 3, 8, 9, 10, 11, 12, 14, 15, 24, 25, 26) melanocytes with giant melanosome complexes, either without a "limiting membrane" surrounded by bundles of microfilaments (Figs. 1 and 2) or with a "limiting membrane" (Figs.

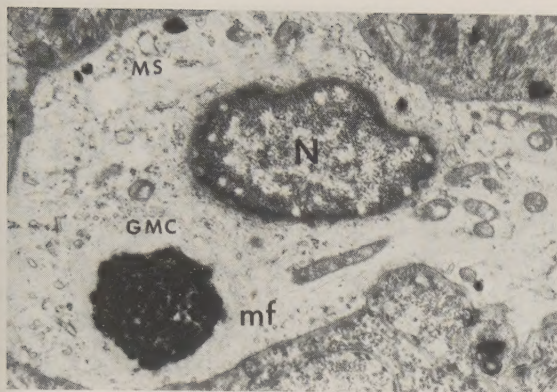


Fig. 1. The epidermal melanocyte. Giant melanosome complex (GMC) apparently without limiting membrane is surrounded by bunches of 100 Å microfilaments (*mf*). (*N*) nucleus. One can see normal melanosomes (*MS*). Patient No. 2; TEM $\times 7550$.

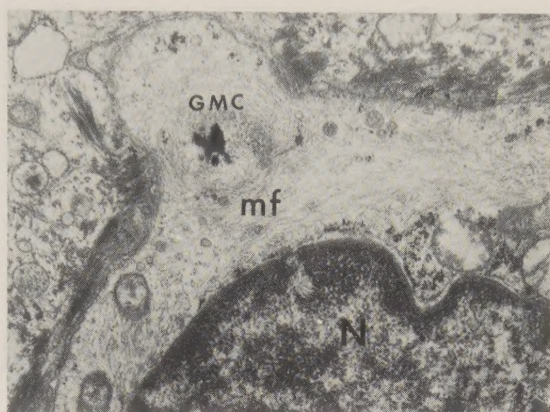


Fig. 2. The epidermal melanocyte. Tangentially cut giant melanosome complex (GMC), apparently without limiting membrane is surrounded by bunches of 100 Å microfilaments (*mf*). (*N*) nucleus. Patient No. 26. TEM $\times 13850$.

3 and 4) could be observed. These cells were most frequently found in the neighbourhood of the malignant melanoma (a few mm and up to 1 cm). Melanocytes with giant melanosome complexes were, however, also found at a greater distance from the tumour, and in two patients in the control biopsies from the opposite leg. In six patients (Nos. 8, 10, 11, 15, 24, 25) melanosome complexes were found lying in fibrocytes and the cells of Langerhans (Figs. 5 and 6), once again most often in the neighbourhood of the tumour. In five patients (Nos. 3, 4, 9, 15, 26) melanocytes with "macromelanosomes" of either globular or fibrillar structure were seen (Figs. 7 and 8). These cells were found at varying distances from the tumour and in one patient (No. 3), on the opposite leg. Patient No. 24, who developed vitiligo around the tumour, had shrunk melanocytes with clear signs of destruction of the inner structure (Fig. 9) in numerous biopsies taken from the vicinity of the vitiliginous area. An increased number of cells of Langerhans and indeterminate cells were seen in these biopsies. In several patients, melanocytes with prominent endoplasmic reticulum, well-developed Golgi apparatus, and an increased number of ribosome residual bodies (Fig. 10) were observed, most often in biopsies removed from the immediate vicinity of the tumour. In a few patients, melanocytes were found with hypertrophic bizarre mitochondria (Figs. 11 and 12). The most remarkable changes

seen in the whole of the melanocyte system were found in patients (Nos. 4 and 26). The majority of the melanocytes studied from these two patients had an increased number of mitochondria and ribosome residual bodies, as well as well-developed Golgi apparatuses and endoplasmic reticula. Patient No. 4 had many melanocytes with suspect "granular-like" melanosomes (Fig. 13). Finally, melanocytes with macromelanosomes were found in both patients. No ultrastructural changes were observed in the melanocyte system in nine patients (Nos. 1, 5, 6, 16, 19, 20, 22, 23, 27) of the 27 in the present material.

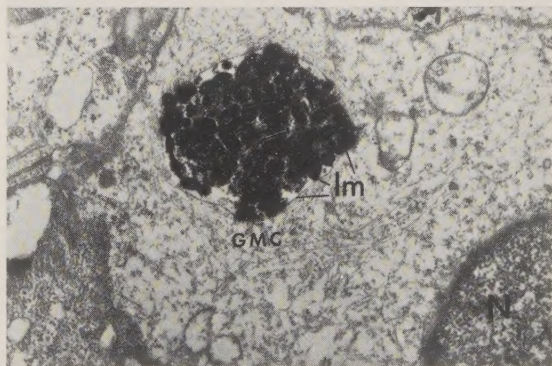


Fig. 3. The epidermal melanocyte. Giant melanosome complex (GMC) with distinct limiting membrane (*lm*). (*N*) nucleus. Patient No. 25. TEM $\times 12350$.

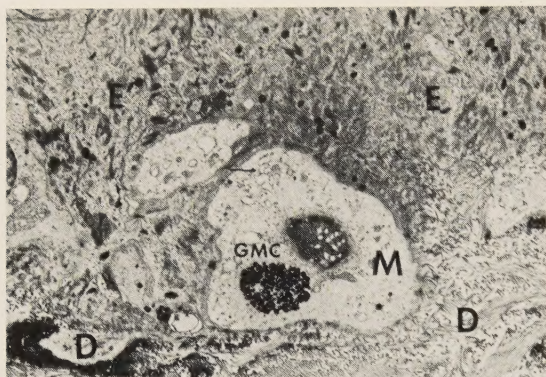


Fig. 4. The basal layer of epidermis. One can see melanocyte (M) with giant melanosome complex (GMC). (D) dermis, (E) basal epidermocytes. Patient No. 10, TEM $\times 3700$.

DISCUSSION

It should be emphasized that malignant cells could not be demonstrated in either the epidermis or the dermo-epidermal junction in biopsies from clinically normal skin around malignant melanoma.

Both qualitative and quantitative changes in the fine structure of the melanocytes have been taken into consideration. The following individual changes in the fine structure of the melanocytes were considered as varying from normal:

1. Giant melanosome complexes—collections of several melanosomes, as a rule, surrounded by a single membrane. The size of these complexes varied considerably.

2. Macromelanosomes—giant organelles, usually of globular structure. However, a variant with fibrillar structure was also observed.

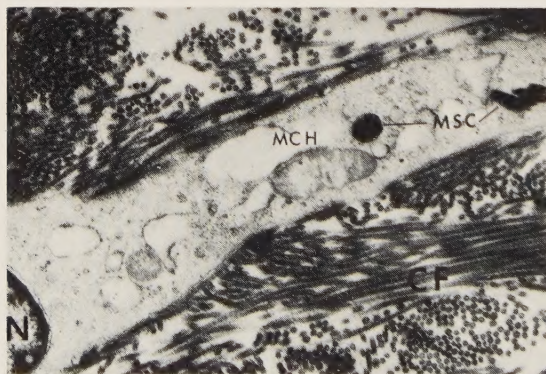


Fig. 5. The dermal fibrocyte. (N) nucleus, (MCH) mitochondria, (MSC) melanosome complexes, (CF) collagen fibres. Patient No. 15. TEM $\times 12350$.

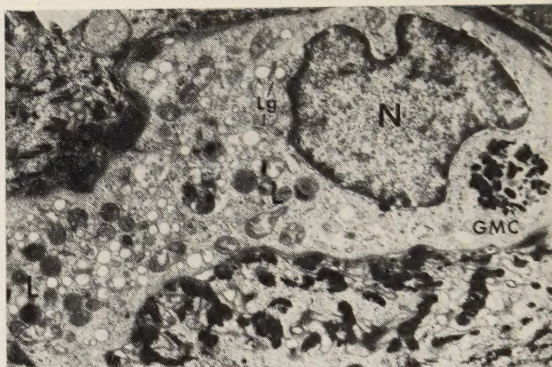


Fig. 6. The Langerhans cell. One can see giant melanosome complex (GMC), typical Langerhans granules (Lg) and lysosomes containing melanosomes (L). (N) nucleus. Patient No. 8. TEM $\times 7200$.

3. Granular melanosomes of varying sizes.

4. Bizarre mitochondria and increased number of lysosome residual bodies.

It is necessary to emphasize the fact that the remaining organelles in melanocytes with the above mentioned variations in the ultrastructure were usually normal.

We have previously demonstrated (Drzewiecki & Drzewiecka, 1979) that epidermal melanocytes with giant melanosome complexes can occur in persons without pigmented skin changes. However, in the present material, these cells were found in 12 of 27 patients, which differs from the normal. Melanocytes with giant melanosome complexes are seen in both hypo- and hyperpigmented changes in the skin, such as Chediak-Higashi syndrome (Zelickson, Windhorst, White & Good, 1967), nevus depigmentosus (Jimbow, Fitzpatrick, Szabo & Hori, 1975), pigmented Mb. Bowen (Kawamura, Ikeda, Mori & Obata, 1966), nevus-melanoma? (Breathnach, 1964) and nevus intradermalis (Szekeres, 1974). The first two changes clearly have a genetic background. The occurrence of giant melanosome complexes in Chediak-Higashi syndrome result from an abnormal tendency to fusion of the melanosomes (Zelickson et al.). On the other hand, in nevus depigmentosus there is an inadequate transfer of melanosomes to the epidermocytes (Jimbow et al.). Giant melanosome complexes in melanocytes in pigmented Mb. Bowen possibly occur as a result of a total blocking of the melanosome transfer to the epidermal cells (Kawamura et al.). UV-light can also result in the forma-

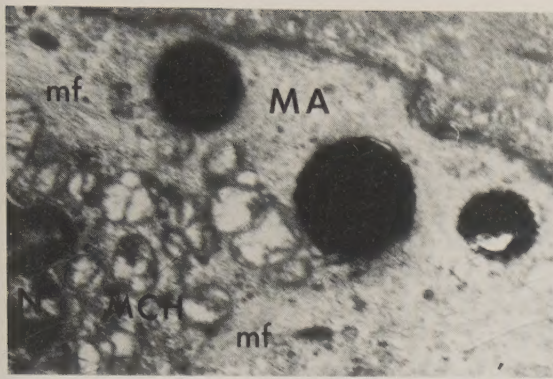


Fig. 7. The epidermal melanocyte with globular macromelanosomes (MA), (N) nucleus, (mf) 100 Å microfilaments, (MCH) swollen mitochondria. Patient No. 9. TEM $\times 12\,600$.

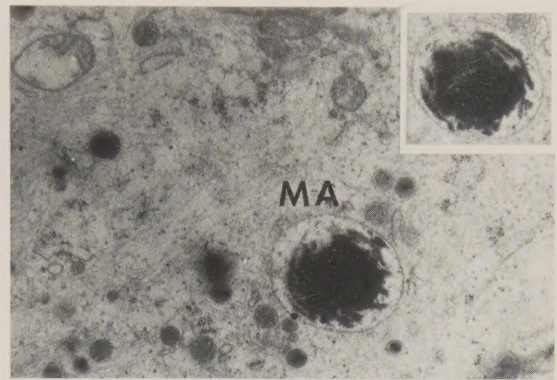


Fig. 8. A part of epidermal melanocyte with fibrillar macromelanosome (MA). Inset: The same macromelanosome, cut in other plane. Patient No. 26. TEM $\times 12\,800$.

tion of giant melanosome complexes in melanocytes. It is considered that an increased melanogenesis is followed by autophagocytosis of the melanosomes, and for this reason, these complexes are formed in the melanocytes (Zelickson, Mottaz & Hunter, 1972). Finally, melanocytes with giant melanosome complexes were found in biopsies from clinically normal skin in patients with metastasizing malignant melanoma (Szekeres & Orfanos, 1978). We may summarize as follows: the occurrence of giant melanosome complexes in melanocytes is a sign of a disturbance in the normal functions of the cell. These disturbances can have either a genetic background, or be caused by some inter/extracellular factors.

Melanocytes with macromelanosomes were found in five patients. The macromelanosomes were observed in a number of hypo and hyperpigmented conditions of the skin with an obvious genetic background, such as: X-linked ocular albinism in both "affected family members" as well as in "carriers" (O'Donnell, Hambrick, Green, Iliff & Stone, 1976), Albright syndrome (Benedict, Szabo, Fitzpatrick & Sinesi, 1968), Xeroderma pigmentosum (Guerrier, Lutzner, Devico & Prunieras, 1973), café au lait spots and neurofibromatosis (Takahashi, 1976; Jimbow, Szabo & Fitzpatrick, 1973; Johnson & Charneco, 1970), and nevus spilus (Konrad, Wolf & Hönigsmann, 1974; Eady, Sparrow & Grice, 1975), multiple lentiginos syndrome (Weiss & Zelickson, 1977) and lentiginos profusa syndrome (Selmanowitz, 1975). The formation of macromelanosomes in melanocytes in the condi-

tions mentioned above appears to reflect a genetically related disturbance in the melanogenesis. Melanocytes with macromelanosomes have been found in nevus-melanoma? (Breathnach) as well as in normal skin from patients with metastasizing malignant melanoma (Szekeres & Orfanos; Konrad & Wolff, 1974). Szekeres & Orfanos put forward the hypothesis that malignant melanoma in the later stages can be accompanied by universal disturbances and increased activity of the epidermal melanocytes. This was the case in one of our patients (No. 26), who had developed many cutaneous metastases shortly before universal spreading of disease. However, four remaining patients had a

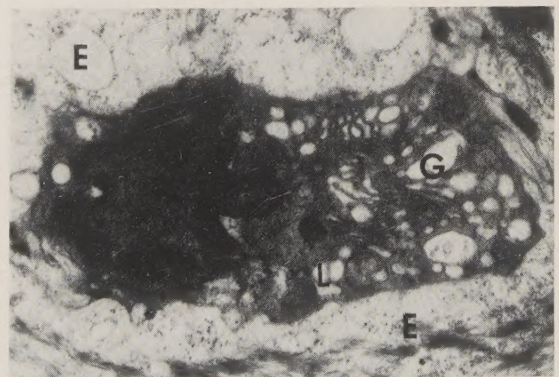


Fig. 9. The shrunk epidermal melanocyte from the vitiliginous area. Golgi apparatus is distended (G), single melanosomes are within big lysosome (L), there are no premelanosomes. The cytoplasm of surrounding epidermal melanocytes (E) is swollen. Patient No. 24. TEM $\times 12\,800$.

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